2293

REPRINTED FROM

TREATISE ON ANALYTICAL CHEMISTRY

PART II

ANALYTICAL CHEMISTRY OF INORGANIC AND ORGANIC COMPOUNDS

VOLUME 11

Part II
Section B-1

ORGANIC ANALYSIS: NITROGEN

By Grant M. Gustin, *Eaton Laboratories, Norwich, New York*, and Clyde L. Ogg, *U.S. Department of Agriculture, Philadelphia, Pennsylvania*

Contents

Contents (*continued*)

Part 2. Kjeldahl Method. By Clyde L. Ogg

I. INTRODUCTION

The two methods most commonly used for determining nitrogen in organic materials are the Dumas and Kjeldahl procedures. Dumas' original method was developed on the macro scale but only the micro adaptation is used extensively today and this is discussed in detail in Part 1 of this chapter. Both macro and micro Kjeldahl procedures are in general use and they are discussed in Part 2. A closed-tube digestion technique applicable to microanalysis and particularly to ultramicroanalysis are also discussed there.

The newest innovation in nitrogen analysis is automation. The Dumas method combines mechanical furnace movements and electrical flow controls through a programmed timer. The Kjeldahl method combines rapid digestion of the sample with a colorimetric measurement of the ammonia formed. These new techniques, discussed in Parts 1 and 3, hold considerable promise for laboratories that need to perform a large number of nitrogen analyses routinely.

Another method which is gaining favor for the analysis of certain materials, particularly petroleum products, is the hydrogen reduction procedure of ter Meulen. Colorimetric instead of titrimetric determination of the ammonia formed in the Kjeldahl digestion is gaining in popularity, particularly for micro and ultramicro determinations. The ter Meulen method and some of the colorimetric methods are described briefly in Part 3.

Gas chromatography measurement of nitrogen is a new and interesting approach to microanalytical combustion methods, but so little has been established yet and the sophisticated electronic instrumentation required is beyond the abilities of a normal laboratory to construct. Several novel techniques of separation and measurement are described in Part 1.

Although the method of choice depends primarily on the chemical and physical nature of the samples to be analyzed, prior knowledge of the sample structure is not as essential with the Dumas method. Samples containing N—N or N—O linkages should be analyzed by the Dumas or ter Meulen method even though many of these materials can be analyzed satisfactorily by modifications of the Kjeldahl method. Materials which

do not contain the above linkages can be analyzed by any one of the three methods.

If more than a few milligrams of material are required because the sample is low in nitrogen content or not sufficiently homogeneous, the Kjeldahl or ter Meulen method should be used. The Kjeldahl method is most suitable for the analysis of samples which are in solution or suspension in water or volatile organic solvents. Modifications and limitations of the Dumas and Kjeldahl methods for the analysis of specific types of materials are discussed in the respective sections. The recent advent of rapid Dumas procedures eliminated the advantage Kjeldahl methods held for multiple analyses on the micro scale. At present the number of analyses one analyst can perform per day is roughly the same for the two methods.

Part 1

Dumas Method. By Grant M. Gustin

I. INTRODUCTION

The measurement of total nitrogen in organic structures according to the ultimate or gasometric method proposed by Dumas in 1831 has become a universally accepted method. Dumas thermally decomposed organic material with a metal oxide in an atmosphere of carbon dioxide. The liberated nitrogen gas was then measured volumetrically at atmospheric pressure over a solution of potassium hydroxide; the solution absorbed all other combustion products and the carrier gas carbon dioxide.

Although Dumas' theory was basically sound and simple, this simplicity was difficult to demonstrate. Pregl's subsequent micro adaptation of this macro procedure greatly improved the accuracy and shortened the analysis time from 3 hours to 1 hour. As a result, micro and semimicro applications have since become the only practical scale for this method; macro scale development has virtually ceased.

But the procedure's apparent simplicity was not attained by Pregl. Great skill in combustion of samples at a slow rate and astute judgment in terminating the sweep period still remained essential. Consequently, its use for routine nitrogen analyses was often unsatisfactory and, in some instances, used only as a last resort after exhaustive attempts by other control methods, notably Kjeldahl, failed.

Until recent years, several inherent sources of error persisted because the factors which created them were not understood. Universal acceptance of

certain ineffective remedies became standard operating techniques that unfortunately retarded the development of simple, rapid, and more accurate Dumas methods. These sources of error merit elucidation.

The serious problem of contaminants migrating into the carbon dioxide carrier gas was often explained as atmospheric nitrogen. Many assumed that rubber tubing connections either absorbed nitrogen from the atmosphere and subsequently released it into the carbon dioxide, or allowed the atmosphere to diffuse through their side walls. Modifications to eliminate the contamination problem were designed with these assumptions. Yet, the problem of impure carbon dioxide persisted as evidenced by the large bubbles rising in the nitrometer at the termination of every analysis. The subjective decisions of an operator to differentiate between the bubbles of nitrogen from the analysis and the impure carbon dioxide following were often difficult if not impossible. Recent studies have revealed the real problem to be volatile components released from rubber, plastics, reagents, and lubricants. Therefore, the corrective measures have since been to use only those materials releasing no significant volatile contaminants. Now, no testing for microbubbles before and after each analysis is needed and this subjective decision previously required of an operator has been eliminated.

An error related to the shrinking volume of nitrogen collected in the measuring chamber of Pregl's nitrometer was erroneously explained as slow drainage of caustic solution from the upper part of the chamber occupied by nitrogen. The resulting classical techniques developed for this "drainage" required a 20 to 30 minute delay before reading the nitrogen volume and an arbitrary 2 to 3% corrective factor subtracted from the observed volume. This factor was found necessary to adjust the unexplainable high results of standard compounds. Again, recent developments have revealed the actual phenomenon to be residual carbon dioxide remaining in the inadequately scrubbed nitrogen. Now, through agitation or greater carbon dioxide dilution of the combustion products, carbon dioxide is completely assimilated in the absorption chamber. Thus, the "drainage" problem has been eliminated and nitrogen measurements are made without delay. Also, the arbitrary correction factor no longer exists.

As more complex and thermally stable compounds were developed over the years, the need for higher combustion temperatures became evident. The resulting complication of dissociating carbon dioxide led to various modifications in packing materials and furnace temperatures. The most significant change was Shelberg's (151) incorporation of a second combustion tube or posttreatment tube. In this, a simultaneous oxidizing and reducing treatment of the combustion products was carried out at a lower

temperature than that used for combustion of the sample. Consequently, much higher and less critical combustion temperatures could be used and, with adequate treatment of the combustion products being assured in the posttreatment tube, rapid combustions were practical.

Today, both the theoretical concepts and simplicity of the Dumas method are a practical reality. Present combustion, absorbing, and measuring technology has overcome most of the inherent limitations that formerly required great skill and subjective judgment of the operator. However, judicious consideration of the fundamental principles and careful selection of proper equipment are essential to maintain the high accuracy, and simplicity, now attainable. Through extensive mechanization (27,48), the method is becoming widely accepted outside the usual research laboratories. It has now become a routine control procedure in many diversified applications such as natural products, medicinal and biological specimens, and industrial products.

II. DETERMINATION

In the thermal destruction of organic materials with the oxidation and catalytic aids of metal oxides, strict adherence to three generalized conditions is required: (*1*) nitrogen complexes must be completely converted to N_2 gas with all other combustion products rendered caustic-soluble, (*2*) reagents and materials of construction in contact with the carbon dioxide carrier gas (>99.999%) must be free of any volatile components, and (*3*) nitrogen gas must be scrubbed free of all other gases by the absorption solution, potassium hydroxide. Only those methods considered to be the most practical to achieve these conditions are described in detail below. Other developments are briefly discussed in Section II-G. A more comprehensive evaluation of all Dumas methods can be elicited from the extensive bibliographies of various books (73,162), reviews (90,145), and annual progress reports (163,167,168) already published.

The most significant developments and commercial applications of the method are in the micro range that involves 1- to 10-mg. samples yielding nitrogen volumes of 0.1 to 1 cc. (0.1 to 1 mg.). The gas is measured to ±0.001 cc. Nitrogen concentrations above 1% are normal, although modifications for 0.1% and less are practical. Excellent precision of ±0.2% absolute accuracy (47) and 0.02%, 0.06% standard deviations (34,161) are reported. Decimilligram (0.1 mg.), semimicro (10 to 100 mg.), and macro scale procedures are essentially modifications of the micro method and are discussed where applicable. Usually the only major changes involve the size of the combustion tube and measuring chamber.

Exceptional care is required in preparing reagents and assembling the entire apparatus. Otherwise, perplexing deviations occur which become difficult to diagnose and rectify. Table IV provides a quick reference to many difficulties with appropriate corrective measures described.

Through automation (48) and commercialization (27) of the Dumas procedure, it is now being used for textiles, plastics, medicinal products, biological specimens (urines, tissues, and body fluids), natural products

TABLE I
Diverse Applications of the Dumas Nitrogen Method[a]

Material[b]	Nitrogen range,[c] %	Special techniques
Cereal and cereal flour	3–13	Co_2O_3 added as catalyst and *ca.* 5-mg. sample size
Dairy concentrate	2–9	Normal techniques
Coffee	25	Normal techniques
Soybeans	6–8	Normal techniques
Tobacco	2–5	Normal techniques
Liquid feed supplement	1	Normal techniques and weighed in aluminum boats
Solid feed supplement	0.3	Co_2O_3 added to 20-mg. samples
Fertilizers	1–45	Normal techniques. Pure inorganic nitrates required addition of organic material, such as benzoic acid, to prevent low results
Urea	45	Normal techniques
Dried rat homogenate	10	Normal techniques
10% rat brain homogenate	0.3	Cooled during preheat period
Lipids	0.3–5	Normal techniques. Less than 20-mg. samples used
Powdered carnation	2–5	Normal techniques
Humus soil	0.1–0.3	50–100 mg. samples used
Lake bottom sediment	0.6	30–100 mg. samples used
Coal	1	Potassium dichromate catalyst added to sample
Shale oil	2	Cooled during preheat period
Oil additives	4–6[d]	Normal techniques
Refinery feedstock	Parts per million	Nitrogen compounds concentrated on silica gel and gel burned
Jet fuel	0.1	Cooled during preheat period
Phosphorous nitride	17	Co_2O_3 added as catalyst
Styrene acrylonitrile	8	Combustion extended 2 min.

[a] Compiled by L. Malter (96).
[b] All materials were tested with the Coleman Nitrogen Analyzer (27).
[c] Relative deviations of 1.5% or less were reported except where noted.
[d] Sulfur appeared to cause low but acceptable results.

(tobacco, cereals, grains, dairy, and food), and commercial products (28, 134) (fertilizers, oil, coal, and fuels). Table I lists many specific applications and, where necessary, the modification used.

A. DECOMPOSITION

The thermal destruction of nitrogen complexes to N_2 gas is carried out in a combustion tube packed with a metal oxide such as copper oxide. This packing serves both as a support for the sample and as a source of oxygen to aid the combustion. A posttreatment tube of copper and copper oxide provides both oxidizing and reducing conditions for the final conversion of nitrogen to N_2 gas and other combustion products to caustic-soluble gases.

Before the sample is pyrolyzed, air is flushed from the system and the copper oxide packing is preheated to 800 to 900°C. on both sides of the sample. Thus, when the sample degrades or volatilizes on combustion, complete thermal breakdown of the structure is assured. Since degradation or volatilization must not occur during the preheat period, cooling of the sample area may be required if the furnaces are close to the sample area. The melting point of a sample is not an indication of its thermal stability, for many substances are known to deteriorate as they melt. Thus low-melting solids, as well as liquids, may need chilling during the preheat period. Sometimes the difficulty of premature pyrolysis is detected before the conclusion of the preheat period when the normal bubbling in the absorption chamber of expanding carbon dioxide does not subside. Persistent or unusually fast bubbling at this time serves to inform the operator that the analysis may be worthless and subsequent samples must be cooled. Drastic chilling with Dry Ice (−70°C.) may be necessary with volatile materials. The preheat period is continued after chilling to raise the combustion tube temperature to normal operating conditions. These techniques are also used when other sample interferences, described in Section II-B-3, are encountered.

Materials that melt or vaporize usually make intimate contact with the copper oxide packing and combust without difficulty. However, the catalytic conversion of their decomposition products to caustic-soluble gases may be required. For materials that release unusual amounts of methane or ethane, the catalytic effect of cobalt oxide is needed. Otherwise, high nitrogen values are obtained. Compounds with several alkoxyl groups within the molecule are known to interfere in this manner.

Some gaseous decomposition products of sulfur compounds are also known to resist pyrolysis and are not readily dissolved in caustic. Their interference is evident by shrinking nitrogen in the measuring chamber.

The operator's first assumption, on observing this shrinking phenomenon, is that a leak has developed in the measuring system. The addition of vanadium pentoxide or potassium dichromate to the sample is needed to destroy these interfering sulfur complexes.

Vanadium pentoxide provides a very effective molten flux and source of oxygen for materials that char or otherwise do not make adequate contact with the copper oxide packing. Pyrimidine, purine, carbazone, diazo, triazo, or pteridine structures are some of the more refractory types of nitrogen-containing compounds (23,161,162) requiring the molten flux treatment of vanadium pentoxide. Some textiles, plastics, and natural products are known to char rather than melt and may require this treatment. Other less rigorous metal oxides and salts (161), such as cobaltic oxide, tungstic oxide, lead chromate, perchlorates, dichromates, and acetates, have also been tested. Hozumi, Imaeda, and Kinoshita reported on the availability of oxygen from various metal oxides (56) and, based on their study, developed a procedure using cobaltic oxide (57). Gawargious and Macdonald described a method for preparing cobalt oxide (43); this preparation has been considered to be the most active form (41).

Combustion tubes are severely attacked by the metal salts which give a molten flux. Cobalt oxide added to these metal salts readily assimilates the flux and thereby keeps it from fusing with the glass. Organometallic samples are also covered in this manner for the same reason. Sternglanz and Kollig (161) packed the sample boat containing vanadium pentoxide in a platinum sleeve to protect the combustion tube.

Samples larger than 20 mg. require, in addition to the oxidation aids of cobaltic oxide and vanadium pentoxide, a slow rate of combustion to assure adequate contact of voluminous combustion products with the hot metal oxide packing. Continuous bubbling into the nitrometer during combustion must not exceed 20 to 30 cc./minute, the same bubbling rate that is observed during the sweep period at the conclusion of the combustion. To minimize the amount of sample needed for trace measurements, such as a few parts per million, nitrogen compounds in oil mixtures have been concentrated on fluorosil (54) and silica gel (96) and the mixture combusted.

Explosive materials having no destructive force are easily analyzed without special treatment. However, materials known to be dangerous explosives are not to be combusted but must be analyzed by a safe means of chemical degradation, such as the Kjeldahl method.

While some inorganic nitrates can be analyzed by the Dumas method, low results may be obtained without the addition of an equal amount of organic material such as benzoic acid or salicylic acid. These organic accelerators must be free of nitrogen. Some inorganic nitrogen structures

can also be successfully decomposed with the addition of the metal oxides mentioned above.

To successfully analyze a variety of materials without having prior knowledge of their structure, a mixture of metal oxides can be added to each sample. A mixture having equal weights of vanadium pentoxide, cobalt oxide, and copper acetate provides a molten flux for the intimate contact needed for charring materials, a catalyst needed for any methane or ethane released from some materials, and a source of additional oxygen needed for refractory materials

B. REMOVAL OF INTERFERENCES

Considerable emphasis is placed on the carbon dioxide carrier gas, its source, and the materials of construction in contact with it. The 50 to 100 cc. of carbon dioxide used to sweep all combustion products into the absorption chamber must not contain any measurable volume of caustic-insoluble impurities. Since the smallest measurable volume in Pregl's nitrometer is 0.001 cc., contaminants must be kept below 0.001%. The indiscreet choice of apparatus, reagents, and combustion techniques can result in significant and sometimes voluminous impurities. Therefore, strict adherence to the following recommendations cannot be overemphasized.

1. Reagents

a. Carbon Dioxide

The only practical source of carbon dioxide is from commercial tanks (800 to 900 p.s.i.). Further purification is required before a tank can be used; however, specially purified tanks are now commercially available at a nominal charge (e.g., Matheson Co., Coleman grade CO_2). Purification in the laboratory is simple and the remaining supply from a 6-pound charge of carbon dioxide will last more than a year in the laboratory (47).

A tank is frozen to −70°C. by storing it in a Dry Ice storage chest overnight. Immediately after removing the tank, its valve is completely opened to blow off the residual pressure of carbon dioxide and gaseous impurities. Condensing carbon dioxide occasionally restricts the valve port. This is dislodged by manipulation of the valve. The purge is stopped before it diminishes to the rate whereby air can diffuse back into the valve. The purge is repeated three or four times after appropriate warmup intervals of 5 to 10 minutes. The tank reaches room temperature in several hours; however, it may be used sooner if necessary. Before attaching a regulator, both the regulator and tank connections must be cleansed of grease or any other foreign material with ether or acetone on a cotton-

tipped probe. The washer seal between the regulator and tank, usually supplied with the tank by the manufacturer, must be a graphite-impregnated fiber washer free of any contaminants.

Before the regulator can be attached to the combustion apparatus, air entrapped in the coiled tubes of the pressure gages must be purged to the atmosphere. Purging is quickly accomplished by temporarily opening the tank valve to fill both pressure dials with carbon dioxide. After the tank valve is closed, the high pressure of carbon dioxide in the regulator is vented to the atmosphere. This filling–venting procedure is repeated four to six times to insure the complete removal of air. If it is not properly purged from the pressure dials, air will slowly diffuse into the gas stream and appear as high blanks in the nitrometer that diminish slowly with subsequent blank tests.

The stem packing on tank valves usually gives off small amounts of contamination that can accumulate to a significant amount, but this is prevented by bleeding carbon dioxide to the atmosphere at a slow rate of 1 to 5 cc./minute. When a tank has been closed for several days or more, the accumulation of contaminants is purged in the manner just described above.

The insides of pressure-reducing valves are usually free of grease, rubber, plastic, and other sources of contamination; however, the needle valve commonly used to control the exit flow of carbon dioxide must be degreased. The needle valve must be removed from the regulator, taken apart, degreased with ether or acetone, dried of solvent, and reassembled using only the pipe thread sealant recommended in Section II-B-2.

If commercial tanks of carbon dioxide are not available, alternate reservoirs, such as Dry Ice-packed Dewar flasks or Kipp generators, can provide carbon dioxide of comparable purity but in limited quantity. Special techniques of packing the Dewar flask and Kipp generator have evolved (162) from the many years of development. However, several precautions must be exercised to maintain the purity of carbon dioxide. Only neoprene-type stoppers and tubing can be used and any necessary lubricant or sealant must conform to the requirements in Section II-B-2.

b. Tube Packings

Solid reagents used in the combustion and posttreatment tubes must not release any contamination when heated. Copper oxide, the universal oxidation packing for supporting the sample in the combustion tube, is ignited in a muffle oven at 800 to 900°C. for several hours to remove contaminants. Copper oxide finer than 60 mesh is discarded, as it restricts the gas flow and fuses. Packings discharged from the combustion tube

after each analysis are collected for subsequent ignition before being reused. This is to reoxidize partially reduced material and remove halogens, sulfur, and other elements which have reacted with the packing. These elements, if allowed to remain and accumulate, eventually contaminate carbon dioxide. A cool area of copper oxide packing in the combustion tube must extend at least 30 to 40 mm. beyond the furnace area toward the nitrometer to provide a condensing area for these interferences. Otherwise, they pass into the nitrometer and give high nitrogen results.

Even when ignited and stored in a carbon dioxide atmosphere, copper oxide still gives a contamination of 0.004 to 0.008 cc. in a blank analysis. Since this "blank" remains constant (±0.001 cc.), the simplest expedient is to subtract the "blank" from the observed nitrogen volume.

Other reagents added to samples as oxidation aids must not yield any contaminants and are checked with a blank analysis. Prior ignition at a nondestructive temperature may be necessary to remove interferences.

Copper from turnings, screening, or wire must be degreased and ignited at 600 to 700°C. in an inert stream of nitrogen or carbon dioxide. It must be cooled in the inert atmosphere. Copper made by reducing copper oxide wire (between 20 and 60 mesh) in a stream of hydrogen or methyl alcohol vapors requires the same ignition and cooling treatment. This latter wire form has a much greater surface area because of its rough porous surface and therefore a greater capacity in the posttreatment tube. However, it tarnishes and becomes inactive from exposure to moisture in the air; therefore, it must be stored with a desiccant.

Heating copper oxide to 800 to 900°C. in the combustion tube, essential for the complete pyrolysis of many materials, results in partial dissociation of copper oxide and carbon dioxide to caustic-insoluble oxygen and carbon monoxide. If these dissociation products are allowed to pass into the nitrometer, they appear as nitrogen. Pregl's single combustion tube does not provide both oxidizing and reducing zones at the optimum temperature (400 to 600°C.) necessary to remove these interferences except with lower furnace temperatures and extremely slow movement of the gas stream during the pyrolysis of a sample. Such techniques are time consuming and difficult to maintain. Shelberg's (151) two-unit combustion system, with copper oxide and copper in a posttreatment tube at 400 to 600°C., provides for both absorption of oxygen and conversion of carbon monoxide to carbon dioxide even with fast flow rates in excess of 50 cc./minute. Since air will prematurely exhaust the hot copper packing, special precaution is taken to purge air from the posttreatment tube with carbon dioxide before the tube is heated. Failure to purge air from a combustion tube at the start of an analysis will have the same effect. High blanks obtained after repacking

the posttreatment tube indicate contaminants in the Cu–CuO reagents. The contaminants are easily expelled by heating the tube at 700°C. for 15 minutes while purging with a slow flow of carbon dioxide. Exhaustion of this packing is accelerated by halogens and sulfur. Therefore, the frequent analyses of compounds containing these elements necessitates a daily check on the length of bright copper remaining. High, erratic blanks or results (0.010 to 0.050 cc.) indicates this packing is exhausted and must be replaced even when there is no visible indication of deterioration. A sudden failure to get all nitrogen may indicate the copper is exhausted whereupon caustic-soluble nitrogen oxides reach the nitrometer.

An alternate packing of hopcalite or manganese dioxide at 100 to 150°C. in the posttreatment tube effectively removes dissociation interferences but it also absorbs nitrogen oxides and slowly releases oxygen. This, therefore, necessitates the use of hot copper (400 to 600°C.) both preceding and following it. While this arrangement is effective, it is less practical in terms of the additional heaters and electrical controls needed.

c. Absorption Solution

The caustic solution of 50% potassium hydroxide in distilled water has a tremendous capacity for both carbon dioxide and the soluble carbonate reaction product. Only reagent grade potassium hydroxide, free of sodium salts, is used. The presence of sodium is obvious from the immediate appearance of an insoluble milky carbonate precipitate when carbon dioxide bubbles into the solution. This precipitate not only prevents adequate scrubbing of the nitrogen bubbles but causes foaming at the meniscus such that a measurement of the nitrogen volume is impossible. Some foaming of potassium hydroxide also occurs in the Pregl nitrometer as caustic in its measuring chamber becomes exhausted. Residual carbon dioxide in nitrogen, inadequately scrubbed in the absorption chamber below, quickly exhausts caustic at the meniscus. Dorfman *et al.* (34) replenished caustic in the measuring chamber by expelling at least 5 ml. up into the nitrometer funnel after each analysis.

The tremendous heat evolved on dissolving potassium hydroxide must be dissipated before the solution is added to the nitrometer. Sediment must be filtered from the solution. Usually, excessive sediment develops in caustic added to new or clean nitrometers. This must be removed by several rinses with caustic before the nitrometer is filled. When nitrometers are drained of spent caustic, no rinsing with water is attempted before refilling with fresh caustic to avoid the development of sediment.

Nitrometers do not need refilling every day. About 50 to 60 analyses

for 100 ml. of caustic is a practical limit. Pregl nitrometers, with much less caustic volume, have a proportionately smaller limit.

2. Equipment

a. Combustion Assembly

All stopcocks, connections, and valves must be bubble-tight and free of any volatile material that can contaminate the carbon dioxide carrier gas. Most rubber, plastic, and lubricating materials fail to meet this latter requirement of no contaminants. Perplexing and exasperating difficulties result with the indiscriminate choice of even one item.

Only one type of lubricant has been found free of contaminants. Apiezon grease, a petroleum by-product of extremely low vapor pressure, contains no plasticizer or additives commonly used in other high-vacuum lubricants. A product of Apiezon Products, Ltd., distributed through James Biddle Co., Philadelphia, Pennsylvania, it is available in several consistencies. Grade N, safely used to 30°C., is suitable for cold stopcocks and seals, but Grade T, which can be safely used at a much higher temperature of 100°C., is used where seals are subjected to heat. These lubricants must be used on all types of metal pipe thread and compression fittings since dry fittings cannot be kept bubble-tight. Any disassembled or loosened fitting must be regreased before tightening. Kroenig cement can be used for rigid seals, because it contains no contaminants.

Neoprene or a similar type of synthetic rubber is free of the voluminous interferences present in vulcanized rubber. No other rubber product has been found acceptable to date. Heavy-walled neoprene tubing is necessary for bubble-tight connections. Neoprene-type stoppers must be used in the end of the combustion tube. However, when unduly exposed to hot furnaces, neoprene degrades and frequent replacement then becomes necessary to avoid interferences from the degradation products.

Neoprene plunger inserts of electric solenoid valves, used in programmed nitrogen analyzers (27,34,48), eventually weaken and require replacement. For convenience, the entire plunger is replaced; the manufacturer must be consulted for proper size.

Clamped ball and socket joints are more flexible and easier to assemble. Similar joints on combustion tubes are also practical, but the increased fabrication costs for quartz or Vycor may not justify their use. All joints must be sealed with Apiezon grease described above.

No parts for the assembly can be considered clean until they have been completely disassembled, degreased with ether or acetone, dried, and resealed with Apiezon grease. This treatment is necessary with metal valves,

solenoid valves, stopcocks, flowmeters, tubing (glass, metal, and neoprene), metal pipe-tubing fittings, and ball-and-socket joints. Soldered tubing must be cleaned of any flux.

Cleaned combustion tubes and posttreatment tubes still release contaminants when first heated. Therefore, their entire length is passed slowly through a hot gas flame to remove all but the last traces of interferences that come off during the first combustion. The first blank obtained with a new combustion tube will invariably be high.

Clean glass or quartz wool retaining plugs are inserted into the tubes without special treatment.

Sample boats are cleaned before they are used. Platinum or porcelain boats are boiled briefly in 1:1 nitric acid and ignited in a gas flame after each analysis. Aluminum foil boats, good for only one analysis, cannot be cleaned by ignition in a flame. However, traces of grease and other contaminants are adequately removed by soaking and rinsing the boats in reagent or spectral grade solvents (acetone or chloroform) and drying at 100°C. for several hours. Cleaned boats are stored in a covered container.

b. Absorption-Measuring Assembly

Nitrogen bubbles must be completely scrubbed of all other gases before entering the measuring chamber. The simple absorption chamber of Pregl's nitrometer (see Fig. 1) does not meet this requirement if the nitrogen concentration in the gas stream exceeds 1 to 2%. As bubbles rise, carbonate shells that interfere with further scrubbing immediately form around them. Bubbles with more than 1 to 2% nitrogen do not shrink sufficiently to distort and break the carbonate shells. Subsequently, they rise too quickly into the measuring chamber while retaining carbon dioxide. This residual carbon dioxide is indicated by the shrinking nitrogen volume in the measuring chamber.

This problem is eliminated by first diluting combustion products with additional carbon dioxide in the Zimmerman mercury-filled reservoir (184) located next to the nitrometer. During combustion, gases are collected in the reservoir and then diluted with carbon dioxide to 1 to 2% or less nitrogen. When transferred to the nitrometer, the bubbles shrink sufficiently to break their carbonate shell and also rise more slowly. Thus, adequate scrubbing in Pregl's absorption chamber is achieved.

The recent development of an agitated absorption solution (27,48) eliminates the need for diluting combustion products in a Zimmerman reservoir. Rapid agitation of the caustic and bubbles with a magnetic stirring bar (see Fig. 2) provides adequate scrubbing regardless of nitrogen concen-

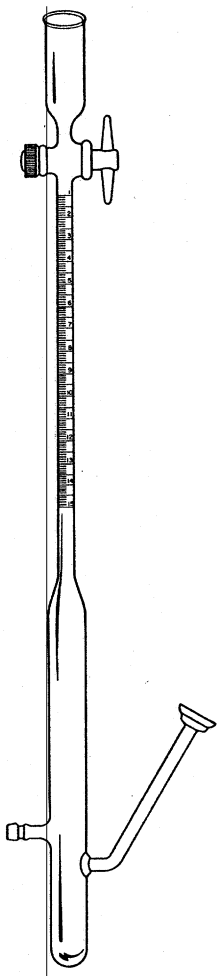

Fig. 1. Pregl nitrometer with Teflon plug. (Courtesy Fisher Porter Co., Warminster, Pennsylvania)

tration in the gas stream. Two measuring chambers (see Fig. 3) were developed (47,48) for agitated absorption systems, since nitrogen bubbles collect below the capillary constriction of the measuring chamber. Pregl's nitrometer does not allow drainage of caustic from the measuring chamber when bubbles collect below this capillary.

These measuring chambers have a distinct advantage, for they do not contain caustic and are separated from caustic in the absorption chamber below by a buffer zone of air. This buffer air also keeps caustic away from

the stopcock used for venting nitrogen after its measurement, thereby eliminating the chronic problem of Pregl's nitrometer where caustic seeps down into the measuring chamber from the funneled reservoir above.

Various closures for the Pregl nitrometer are described by Kirsten (73) and a Teflon plug that requires no lubrication is shown in Fig. 1. The problem of seepage in Pregl's nitrometer is simply eliminated by never raising caustic to reach the venting stopcock and keeping at least 0.1 to 0.2 cc. of nitrogen in the measuring chamber at all times. This necessitates an initial reading of the caustic level before an analysis. Any stopcock grease can be used to seal the nitrometer stopcock when it is not wetted with caustic. Contaminants from a grease that would interfere in any other part of the combustion system do not exert sufficient vapor pressure to alter the small volume of nitrogen being measured. However, to avoid the accidental use of such a grease in the other parts, Apiezon grease is recommended for this stopcock as well.

Nitrogen bubbles frequently adhere to the surface of mercury covering the inlet capillary of Pregl's nitrometer. The addition of powdered mercurous chloride (162) or other means of making the mercury dirty temporarily eliminates this problem, but a more positive means of dislodging bubbles is to place a small 10- to 20-mm. long steel wire which can be moved around with a magnet on the mercury surface. Nitrogen bubbles also adhere to the plastic-covered stirring bar in agitated absorption chambers. Full-speed agitation of the stirrer quickly dislodges them. All bubbles must be dislodged before the nitrogen volume is measured.

The measuring chamber must not be exposed to unusual heat sources or temperature fluctuations. If necessary, shielding can be placed around the absorption-measuring assembly to provide the stable temperature conditions needed. Thus, a measurement of the air temperature adjoining the measuring chamber is sufficient to indicate the nitrogen temperature inside, a necessary measurement in calculating the weight of nitrogen.

The collected nitrogen gas is not dry but contains a significant volume of water vapor from the caustic solution. This volume, about 1% of the total volume, varies both with the caustic concentration and the nitrogen temperature. Table III(*a*) shows the partial pressure exerted by water vapor. This is simply subtracted from the barometric pressure.

The rubber-covered inlet of the agitated absorption chamber shown in Fig. 2(*a*) is a one-way valve, plugged at one end and slit along the side. Only natural rubber has the necessary resiliency to simultaneously allow gases to bubble out and prevent caustic from entering. Although natural rubber introduces contaminants into the gas stream, soaking and kneading the rubber in caustic beforehand minimizes this problem.

When not in use, nitrometers must be disconnected from the combustion assembly to avoid the possible backflow of mercury or caustic past the inlet. If caustic is permitted to go back into the capillary inlet and combustion system, the affected parts must be disassembled and cleaned before the apparatus can be used again.

Carbonate deposits often accumulate in the capillary inlet and eventually restrict or block the flow of gases. In this event, the absorption chamber must be disconnected, rinsed free of caustic, and unplugged with dilute acid.

3. Samples

In addition to interferences from incomplete combustion, described in Section II-A, samples must not have occluded air. Occlusions found in materials, such as sponges or foams, must be broken apart. Resilient materials that are difficult to break or grind may be made friable by chilling with Dry Ice. Moisture condensed on chilled materials must be removed with suitable drying conditions.

Rubber, plastics, grease, or any other samples that contain plasticizers or other volatile components will contaminate the carbon dioxide carrier gas. The vapor pressure of these samples may be lowered by chilling the sample-packed combustion tube with Dry Ice during the purge and preheat periods.

Repetitive analyses of aqueous solutions will dilute the caustic absorption solution unnecessarily. Vermiculite in the cold exit end of the combustion tube has been suggested (96) to avoid this possibility. The absorbent is first muffled at 850°C. for 15 minutes and then, after each analysis, is expelled from the combustion tube. Mizukami and Miyahara (108) dried combustion products with magnesium perchlorate.

The analysis of fluorinated compounds results in plugging of the nitrometer inlet from volatile silicon tetrafluoride hydrolyzing with water condensate. Sodium fluoride in the exit end of the combustion tube at 180°C. has been used (13,67) to remove this interference. The use of heated magnesium oxide has been suggested also (41).

C. REAGENTS

1. Carbon Dioxide

Carbon dioxide gas is used to purge the atmosphere from the combustion tube and to push combustion products into a caustic absorption chamber where nitrogen, released from the organic sample, remains as the only gas. Exceptionally pure carbon dioxide (99.999%) is required, and the only

practical source is from commercial tanks that are pretreated to remove impurities (see Section II-B-1). High pressure from the tank is reduced to 3 to 5 p.s.i., which only a two-stage reducing regulator can properly maintain during the rapid flow (200 to 300 cc./minute) of gas. The normal precautions in handling and storing high pressure (800 to 900 p.s.i.) tanks are to be observed such as storing away from any unusual heat sources.

The classical sources, Dry Ice packed in a Dewar flask and carbonate–acid reagents in a Kipp generator, provide carbon dioxide of the same high purity as a tank. However, they have only a limited reservoir that is only slowly replenished; this is inadequate for the rapid Dumas methods now in common use. Their use is recommended only if the tank source is unavailable. In this case, the rapid procedures are slowed during the purge and sweep periods.

Regardless of the size Dewar flask used, its charge of Dry Ice lasts only 7 to 10 days. Tank reservoirs can be expected to last more than a year without further treatment.

2. Copper Oxide

Copper oxide granules are packed into the combustion tube around the sample. They provide a source of oxygen to aid the combustion and a support to hold the sample in place. The commercial "wire" form is usually too coarse and therefore is ground to pass a 20-mesh but not a 60-mesh sieve. With reasonable distribution in size, a uniform dense packing is obtained in the combustion tube. After grinding and sieving, the reagent is ignited in a muffle oven at 800 to 900°C. for several hours to remove volatile interferences (see Section II-B-1) and stored in a clean capped bottle. Activating copper oxide in a stream of oxygen during ignition (64,66) has been recommended (161). It is reused many times but must be ignited each time. Only that portion packed around a disposable aluminum sample boat in the combustion tube is thrown away after the analysis. Methane is known to resist pyrolysis even at high temperatures (63), and its oxidation by various metal oxides has been studied (65).

3. Oxidation Catalysts

Copper acetate, vanadium pentoxide, and cobalt oxide are added to samples requiring additional oxygen and catalytic treatment (see Section II-A). Reagent grade copper acetate crystals need to be ground to a powder. The best quality vanadium pentoxide powder commercially available is used without further treatment if no volatile interferences are released on combustion. It may be heated in a muffle furnace at 400 to

500°C. if necessary. Only one form of cobalt oxide powder is considered active (41) and is prepared, according to the method of Gawargious and Macdonald (43), by precipitating cobalt oxalate from a mixture of a cobalt salt and oxalic acid. After thorough rinsing and drying, the precipitate is first charred at 350 to 400°C., to decompose the oxalate structure, then ignited at 550 to 600°C. for several hours.

A mixture of these three metal oxides in equal parts by weight provides a very effective combination to be used routinely on all samples whenever a variety of structures is handled.

4. Copper

To remove the interference of oxygen, copper provides the reducing conditions needed. Bright copper chips, granules, turnings, or wire must first be degreased (see Section II-B-1) with ether, then ignited in a carbon dioxide atmosphere at 600 to 800°C. to facilitate the removal of volatile contaminants. Granules reduced from the copper oxide reagent described above are more reactive because of the porous surface. The reduction is carried out in an atmosphere of hydrogen or methyl alcohol with heat. While cooling, the reduced copper must be protected from atmospheric oxygen by nitrogen or carbon dioxide. This copper must be stored over a desiccant to protect it from the tarnishing effect of atmospheric moisture.

5. Potassium Hydroxide

A 40 to 50% potassium hydroxide solution is used in the absorption chamber to remove carbon dioxide and other gases from the nitrogen bubbles. Reagent grade solutions of 45% potassium hydroxide are commercially available and require no filtering or other treatment. Solutions prepared by mixing equal weights of distilled water and solid potassium hydroxide (reagent grade, 85% assay) makes a 42 to 43% concentration that may require filtering if sediment develops. The resulting hot solution must be cooled to room temperature before it is added to the absorption chamber.

6. Lubricants and Cements

The only stopcock lubricant presently suitable (see Section II-B-2) is Apiezon grease available in Grade N for cold application not to exceed 30°C. and Grade T for temperatures not to exceed 100°C. All stopcocks, ball-and-socket joints, pipe fittings, and valves are sealed with this type grease.

Kroenig cement may be used for rigid joints.

D. COMBUSTION ASSEMBLY

The combustion assembly, with stopcock, valves, furnaces, and combustion tubes, is rigidly mounted to provide a stable system. For safety and convenience, both combustion and absorption assemblies are secured to a common platform. The sample furnaces are movable in two directions, along the combustion tube and back away from it. Combustion tubes are removed after each analysis and, therefore, are mounted for quick, easy removal. Except for the preassembled vertical combustion system of the Coleman Nitrogen Analyzer (see Fig. 6), the entire system is assembled on a horizontal plane parallel to the edge of the bench as shown in Figs. 7 and 8.

1. Combustion Tubes

Commercially available Vycor or quartz combustion tubes (10 to 11 mm. O.D., 525 mm. long) with capillary tips can be used for both the combustion tube (800 to 900°C.) and posttreatment tube (400 to 600°C.) although, for the latter, Pyrex tubes will hold up at the normal 500°C. temperature used.

Since the automatic Coleman Nitrogen Analyzer has special mounts to secure combustion and posttreatment tubes, only the quartz combustion tubes and Vycor posttreatment tubes supplied by the manufacturer are used on this instrument. The ends of these tubes are specially ground. and polished to maintain a tight seal in the gasketed compression seals. Chipped ends will result in leaks and such tubes must be discarded.

For speed and convenience, several combustion tubes are put into use at the same time. While one is in use and a second is cooling from the previous analysis, a third tube can be packed for the next analysis. A fire-resistant reference ink or mark is applied to the sample area of the tubes. This area is midway between the combustion furnaces. Volatile contaminants are burned out of the tubes at 800 to 1000°C. by simply heating the empty tubes in a gas flame. Then a compact glass wool retaining plug, at least 1 inch in length, is packed into the exit end. The length of the combustion tube is not critical except it must extend 3 to 4 inches beyond both furnaces. Thus connectors and stoppers are not exposed to excessive heat. When exposed to excessive heat radiations, such as the upper combustion tube support of the Coleman Nitrogen Analyzer, connections must be protected with a reflective shield, and frequent replacement of the rubber gasket is required.

The posttreatment tube, 10 to 11 mm. O.D., is made sufficiently long to extend at least 2 inches beyond its 400 to 600°C. furnace in both directions. With a 100-mm. furnace, a tube 200 to 250 mm. in length is adequate.

Copper and copper oxide, densely packed as two separate reagents in equal amounts, do not extend beyond the furnace area, to minimize heat conductance to the tube connections. The reagents are held in place with glass wool plugs. Although there is no data to indicate which reagent the incoming combustion products should pass through first, copper oxide is packed into the fore end of the tube from established custom. Before heating the packing to 400 to 600°C., air must be expelled and not be allowed to reenter, in order to prevent premature degradation of the copper reagent. Refer to Section II-B-2 to avoid this and other interferences.

2. Stopcocks and Valves

The flow of carbon dioxide and combustion products is controlled through stopcocks, metal valves, or electrically operated solenoid-type valves. Although only two three-way stopcocks are used in the assembly of Fig. 7, three check valves, *S-1*, *S-2*, and *S-3*, are also used to provide preset flows of 200 to 300, 1 to 5, and 20 to 30 cc./minute, respectively. Thus, no precise valve adjustments are needed for every analysis and only simple, quick turns of valves *V-1* and *V-2* are required to direct the gas stream.

The check valves are precision flow controls for which either metal needle valves or glass precision flow stopcocks are needed. Valve *S-1* controls the maximum flow rate of 200 to 300 cc./minute into the entire assembly. This rapid flow permits quick purging of the atmosphere from the combustion tube, usually within 40 to 60 seconds. This time must be determined for each assembly. Sternglanz and Kollig (161) recommend a 3 to 5-minute purge and a similar period for the sweep on the Coleman Nitrogen Analyzer. Valve *S-2* is a bleed control (see removal of interferences in Section II-B-1) of 1 to 5 cc./minute that prevents the accumulation of contaminants from the tank valve when carbon dioxide is not flowing through the combustion assembly. Valve *S-3* restricts the flow into the nitrometer to 20 to 30 cc./minute to permit adequate scrubbing. Final adjustments are best made during the blank analysis, since heated reagents restrict the flow.

Valves *V-1* and *V-2* do not restrict the gas stream in any way but serve only as directional controls. Glass stopcocks (120°, 3-way) are used on manual assemblies, while electrical solenoid valves are used in mechanized (34) and automated systems (27,48). Under no circumstances is valve *V-2* turned to connect the hot posttreatment tube, *P*, to the atmosphere. Inadvertent introduction of air quickly exhausts the copper packing and introduces nitrogen into the analysis. This nitrogen must be purged from the system before starting the next analysis. Valve *V-3* in Fig. 8, used with the Zimmerman reservoir–Pregl nitrometer combination, is a glass

stopcock (120°, 3-way) directing the flow of combustion products first into the reservoir, then from the reservoir into the nitrometer. Combustion products remaining in the piping between *V-3* and the nitrometer after the transfer are pushed into the nitrometer by turning the valve to connect the carbon dioxide source to the nitrometer. Any mercury raised into the stopcock plug at the conclusion of the transfer is conveniently pushed into the nitrometer. But careful reopening of valve *S-3* is necessary for mercury to pass.

Before being installed, all valves and stopcocks must be completely disassembled, degreased, and reasssembled with Apiezon grease where seals and lubrication are necessary (see Section II-B-2).

3. Furnaces

Two sample furnaces, at least 75 to 100 mm. long and capable of maintaining 800 to 900°C., are split or opened in a manner to permit their movement away from the combustion tube at the conclusion of each analysis. The furnaces are kept hot between analyses because the speed with which they bring the combustion tube to operating temperature governs the speed of analysis. Although reflective or radiant-type furnaces can attain temperatures of 890 to 900°C., resistance furnaces with refractory insulation have a much greater heat sink that can heat the combustion tube in several minutes. This rate must be established for each type of furnace.

The furnaces are mounted on a stand that allows them to be moved both along the length of the tube and back away from it. If their backward movement is not far enough to avoid premature heating of a combustion tube, a reflective shield must be placed in front of them. Both furnaces are brought together for the combustion and must not be separated by excessive insulation or mounting brackets. The space remaining between them becomes a cool zone in the combustion tube where some decomposed materials condense. This area is heated after the initial combustion period by advancing both furnaces as a unit 25 to 50 mm. toward the exit end of the tube. This final combustion continues for 1 to 2 minutes or until bubbling ceases in the nitrometer.

Gas burners provide rapid heating that facilitates the combustion. However, greater precautions are necessary to shield the nitrometer from such excessive heating.

The furnace surrounding the posttreatment tube is securely mounted and maintained at a temperature of 500°C., a practical working average between the 400 and 600°C. required. It is at least 100 to 150 mm. long for heating a minimum packing of 100 mm., 50 mm. of copper, and 50 mm. of copper oxide.

E. ABSORPTION AND MEASURING ASSEMBLY

Pregl's micronitrometer, despite various modifications of the stopcock (73), has remained basically the same. Ineffective scrubbing is encountered in this static absorption chamber when the nitrogen concentration of entering gases is greater than 1 to 2%. In order to insure this dilution, a modification of Zimmerman's mercury-filled reservoir (47) must be incorporated with the nitrometer.

To circumvent the mercury reservoir, an agitated absorption chamber (27,48) has been developed recently that provides thorough scrubbing of nitrogen bubbles without dilution. Thus, much faster combustion and flow rates are possible. The simultaneous development of other measuring chambers (47,48) has resulted in faster, more accurate, and less troublesome measuring systems. For these reasons, agitated absorption chambers as shown in Fig. 2 and measuring chambers as shown in Fig. 3 are recommended.

1. Agitated Absorption Apparatus

Violent agitation of the caustic solution, provided by a plastic-covered magnet and stirring motor, is necessary to insure the complete removal of all caustic-soluble gases. Caustic does not go into the measuring chamber but is kept below the reference line *R* in Fig. 2. Since the caustic level does not vary with each measurement, a rigid leveling arm is used instead of the adjustable bulb needed with the Pregl nitrometer. The gas inlet capillary *I* is sealed either with mercury (e.g., chamber *b*) or a slitted rubber valve that directs bubbles down onto the stirring bar (e.g., chamber *a*). Only natural rubber has the resiliency necessary for the slitted valve to give a tight seal which 1 to 2 p.s.i. pressure can open without caustic migrating back through it. It is conveniently made from a small rubber policeman with a 5 to 10-mm. slit cut lengthwise in a plane perpendicular to the sealed tip. If not cut in this plane, the seal will not tighten when negative pressure occurs within the inlet, but will open and caustic will go back into the combustion assembly. Because of the backflow problem, a check valve on chamber *b* has been incorporated to halt further migration of mercury. This problem is not encountered during routine analysis with clean absorption chambers, but the chamber must be disconnected at the same time the apparatus is turned off.

Because violent agitation is required, plastic-covered magnets are used to avoid possible breakage. Some nitrogen bubbles adhere to the plastic after each analysis and must be dislodged. This is done by rapidly spinning the magnet after the other bubbles have risen. Bubbles do not adhere as tenaciously to Kel-F type plastic as they do to Teflon. The chamber's base

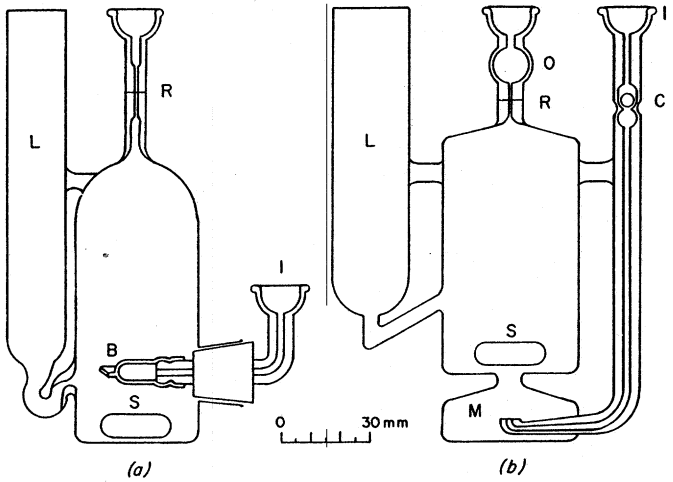

Fig. 2. (*a*) Agitated absorption chamber with slitted rubber inlet (47). *B*, natural rubber policeman with slit underneath; *I*, gas inlet; *L*, rigid caustic liquid leveling arm; *R*, reference mark for caustic level; *S*, plastic-covered magnet. (*b*) Agitated absorption chamber with mercury-covered inlet (27) (courtesy Coleman Instrument Co., Maywood, Illinois). *C*, check float to stop mercury from flowing back into combustion apparatus; *I*, gas inlet; *L*, rigid caustic liquid leveling arm; *M*, inlet chamber filled with mercury; *O*, overflow protection chamber; *R*, reference mark for caustic level; *S*, plastic-covered magnet.

must be flat without the usual glass lump that fabricators leave in flat-bottom glassware. The bottom of chamber *b* has been ground flat to insure undisturbed agitation.

The capillary connecting the leveling side arm *L* to the chamber is above the direct centrifugal forces of the stirring bar and inclined toward the chamber so that bubbles that occasionally find their way into the capillary quickly return.

2. Nitrogen-Measuring Apparatus

Measuring systems as shown in Fig. 3 are required for agitated absorption chambers. They are not filled with caustic as is the Pregl nitrometer. On the contrary, liquid is never allowed to rise into them, but kept down in the absorption chamber at all times by a buffer zone of air. Nitrogen collected in the absorption chamber drops caustic below the reference line *R* in Fig. 2. Caustic is then restored to the line by enlarging the measuring chamber, the volume change being nitrogen.

The mercury-filled column *a* is precision bore capillary tubing graduated to the nearest 0.005 to 0.010 cc. such that 0.001-cc. units can be estimated.

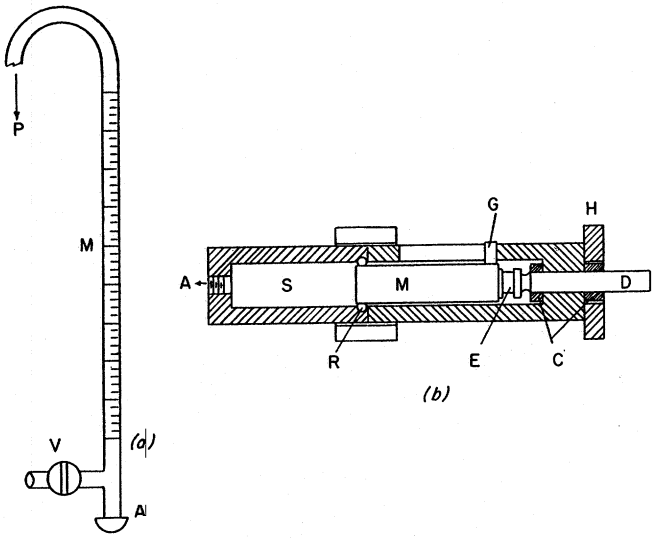

Fig. 3. (*a*) Mercury-filled measuring capillary (47). *A*, ball joint connection to absorption chamber; *M*, measuring capillary with inverted column of mercury; *P*, to piston control filled with mercury; *V*, vent to expel nitrogen. (*b*) Syringe measuring chamber for digital readout (27,48) (courtesy Coleman Instrument Co., Maywood, Illinois). *A*, to vent and absorption chamber; *C*, restraining collars locked onto shaft; *D*, shaft extension to engage digital dial; *E*, micrometer screw; *G*, syringe guide pin; *H*, mounting plate; *M*, precision-ground measuring syringe; *R*, rubber O-ring seal; *S*, syringe cavity.

It is attached to a mercury-filled syringe that raises and lowers an inverted mercury column in the capillary. Practical working heights limit the capillary length to provide only 1 to 2-cc. capacities.

The measuring syringe *b* consists of a metal plunger machined to precise dimensions and geared to a digital counter such that measurements are read directly to 0.001 cc. Because of its compactness, a much larger capacity of 5 cc. is practical. Its various advantages over other measuring systems make it the most satisfactory to use. Elimination of the skill required and fatigue incurred in estimating the volume between capillary graduations is the paramount feature. Small volumes of nitrogen do not need to be vented after each analysis. The final volume of one analysis serves as the initial reference measurement of the next until the syringe capacity is reached. Also, samples unexpectedly giving large nitrogen volumes beyond the 1.5-cc. capacity of other measuring systems are easily handled in the 5-cc. syringe. So prior knowledge of nitrogen content in samples is unnecessary in most applications. However, the complication of tempera-

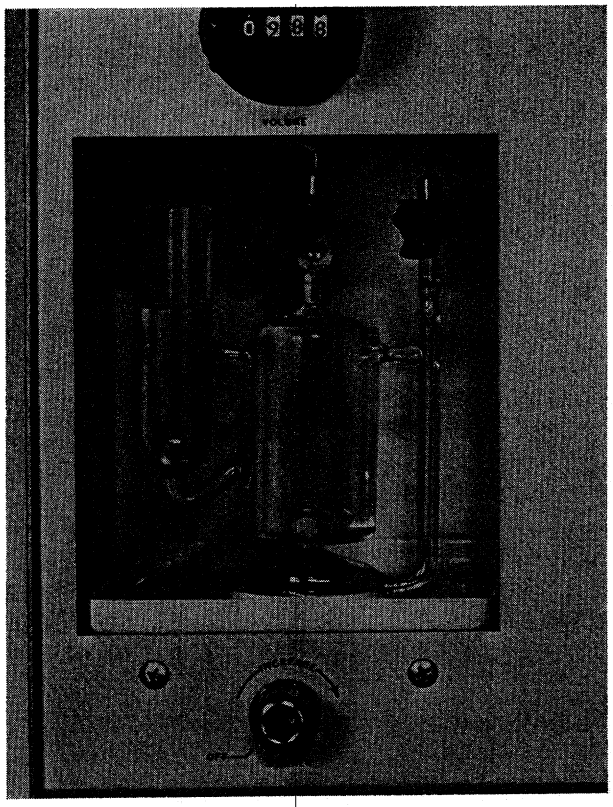

Fig. 4. Mounted absorption chamber (courtesy Coleman Instrument Co., Maywood, Illinois).

ture/volume changes from the large buffer zone of air (*ca.* 4 cc.) surrounding the syringe necessitates a correction of the final nitrogen volume if temperature fluctuations occur between it and the initial reference measurement made before the analysis. Corrections for the final volume are shown in Table II. Because of the large mass of the syringe, nitrogen temperatures within do not respond quickly to air fluctuations; therefore a thermometer well is best clamped around the syringe cavity S for the thermometer to show the same slow response.

Measuring chambers must be shielded from unusual heat radiations and air drafts for measuring the nitrogen temperature (± 0.1°C.). With reasonably stable conditions, the air temperature adjoining the chamber is adequate. Fig. 4 shows an absorption chamber clamped to the measuring syringe within an area protected from air drafts.

Lubricants for the vent V and ball joint A do not have to meet the stringent contamination-free requirements for valves and joints exposed to carbon dioxide, since their partial pressure in the static nitrogen gas is too insignificant to be measurable. Nor do they have to be resistant to caustic solvent action, as with Pregl nitrometers, for caustic does not rise to either the joint or valve. However, the use of Apiezon grease is recommended here to avoid having another lubricant available that might inadvertently be used on other parts of the combustion system. Refer back to Section II-B-2 for the perplexing ramifications that must be avoided.

3. Pregl Nitrometer—Zimmerman Reservoir

The Pregl nitrometer, with various modifications, has been the generally accepted absorption-measuring system until recently (see Fig. 1). However, the static absorption chamber of this type does not adequately scrub nitrogen bubbles except when the nitrogen concentration of entering gases is 1 to 2% or lower (see Section II-B-2).

Therefore, to use this nitrometer, combustion products must be diluted before being passed into the absorption chamber. This dilution is simply carried out in a modification of the Zimmerman mercury-filled reservoir where all gases are collected during the combustion and sweep periods (see Fig. 8). The reservoir has a 60 to 100-cc. capacity and is connected by rubber tubing to an adjustable leveling bulb of similar capacity. All gases are expelled from the reservoir into the nitrometer through stopcock V-3 by elevating the leveling bulb until mercury touches the plug of the stopcock. Substantial supports must be provided for the heavy mercury-filled equipment, with suitable precautions taken to avoid breakage or otherwise spilling mercury.

The nitrometer has sufficient mercury in the bottom to cover the capillary inlet. The opposite higher side arm is connected by rubber tubing to a leveling bulb that is adjusted to the caustic level in the measuring capillary for atmospheric pressure measurements. The caustic-filled capillary cannot drain properly if large nitrogen bubbles are allowed to collect and form a gas–liquid block in the lower part of the capillary. When diluted in the mercury reservoir described above, the bubbles are relatively small and faster flow rates (20 to 30 cc./minute) are practical. Nitrogen bubbles frequently adhere to the mercury surface in the nitrometer and must be dislodged. A small steel wire, previously inserted in the absorption chamber and manipulated by a magnet, quickly dislodges any bubbles.

Because of its strong solvent action on lubricants, caustic in the stopcock and funnel above the measuring chamber frequently interferes with volume measurements by seeping down into the chamber. Various closures have

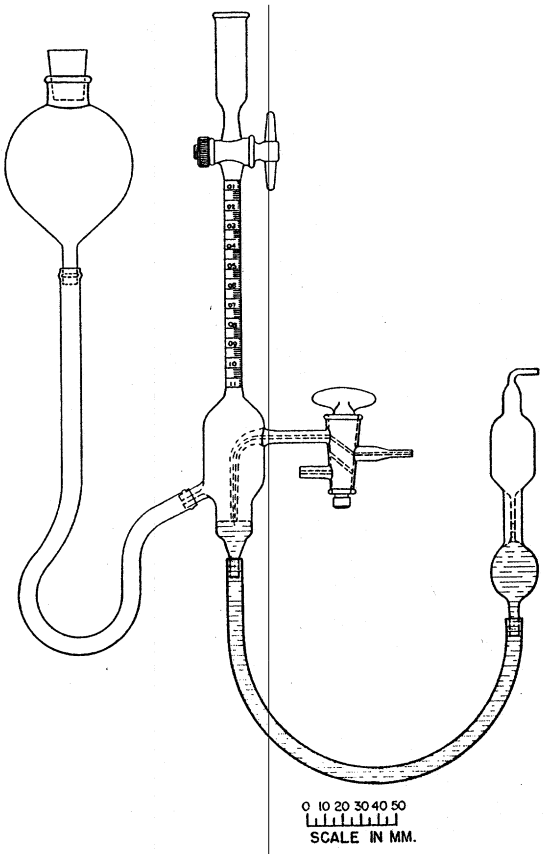

Fig. 5. Nitrometer to impinge gases on lowered mercury surface (34) (courtesy *Analytical Chemistry*, from Vol. 34, p. 679).

been designed (73,108) to circumvent this interference. The Teflon stopcock, shown in Fig. 1, requires no lubrication. Seepage must be forced back up into the closure, without losing nitrogen in the process, by cautiously opening the closure and raising the caustic-filled leveling bulb. This is a difficult, tedious, and often unsuccessful technique.

Two methods of operation are recommended to avoid the seepage problem. The first (73) is to keep the leveling bulb higher than the measuring chamber until nitrogen is measured. When the bulb is lowered to the same level as caustic in the capillary, a brief delay before measurement is needed to allow for drainage from the wet walls.

The second technique, patterned after the buffer air space described in Section II-E-1 for agitated absorption chambers, does not permit caustic to

come in contact with the stopcock. Caustic is raised to the uppermost scale divisions in the capillary with the leveling bulb, the stopcock is then closed, and, with the leveling bulb liquid kept level with the caustic meniscus, the remaining gas volume is read. This reading is subsequently subtracted from the final volume reading to give the actual nitrogen volume. All measurements are made at atmospheric pressure by adjustment of the leveling bulb to the liquid level in the capillary.

A recent modification of the nitrometer inlet (34) shown in Fig. 5 does not require dilution of the combustion products. Mercury lowered away from the inlet, after the carbon dioxide flow has commenced, permits a fine stream of bubbles to impinge upon its surface for agitation. The mercury-filled leveling bulb attached to the base of this nitrometer must be raised before the carbon dioxide flow is stopped, to prevent the obvious flow of caustic back through the inlet.

The analysis of decimilligram and ultramicrogram samples (74,75,79) does not require further dilution of already small nitrogen concentrations.

F. MEASUREMENT AND CALCULATION

1. Nitrogen Gas Measurement

The nitrogen volume is measured to ± 0.001 cc. at atmospheric pressure by adjusting caustic in the leveling side arm to the level of the meniscus in the measuring system. The side arm, or leveling bulb, is never stoppered but exposed to atmospheric pressure. The temperature of nitrogen must be observed to $\pm 0.1°$C. immediately after the volume is observed. Air temperature adjoining the nitrometer is sufficient when no unusual temperature fluctuations are experienced by the nitrometer. If this occurs, shielding of the nitrometer is necessary.

Nitrogen in the mercury-filled measuring chamber (see Fig. 3(*a*)) is the difference between the initial mercury level before analysis and the final level when caustic is again restored to the reference mark R in the capillary of the absorption chamber (see Fig. 2). The small buffer zone of air does not change in volume with small temperature variations between the initial and final measurements. However, the large buffer zone of air in the syringe-type measuring chamber (see Fig. 3(*b*)) does change. A volume/temperature expansion chart, such as Table II must be consulted to apply the appropriate correction to the final reading of the digital counter.

Observed readings in a precalibrated nitrometer are corrected according to the calibration chart supplied by the manufacturer. Stehr (160) described a method of calibration that used nitrogen gas.

TABLE II

Typical Syringe Volume–Temperature Expansion Corrections (27)

Final digital counter reading, cc.	Volume expansion correction,[a] cc./°C.
0.0	0.013[b]
0.5	0.014
1.0	0.016
1.5	0.018
2.0	0.020
2.5	0.021
3.0	0.023
3.5	0.025
4.0	0.026
4.5	0.028
5.0	0.030

[a] Volume correction (V_E) is multiplied by the temperature difference between initial (t_I) and final (t_F) syringe temperatures [$V_E \times (t_F - t_I)$] and subtracted from the final counter reading. (Note: When final temperature t_F is less than t_I, the correction is added to the final reading).

[b] Volume of buffer air in empty syringe is sufficient to show a volume change in spite of a zero reading on the digital counter.

TABLE III

(a) Pressure Correction for Water Vapor over 50% Potassium Hydroxide (106)		(b) Barometer Brass Scale—Mercury Expansion Correction (84)	
Nitrogen temperature, °C.	Vapor pressure,[a] mg. Hg	Barometer temperature, °C.	Pressure correction,[a,b] mm. Hg
15	4.1	15	1.8
20	5.7	20	2.3
25	7.4	25	2.9
30	9.6	30	3.5
35	12.5	35	4.1

[a] Correction to be subtracted from barometric pressure.

[b] Corrections are for 740 mm. atmospheric pressure; however, they do not vary more than ±0.2 mm. between 700 and 780 mm. pressures.

The nitrogen volume must be corrected for small blanks inherent in the combustion system. These are constant and range between 0.002 and 0.010 cc. Although larger reproducible blanks can be tolerated, the sources of contamination should be located and eliminated for the greatest

sensitivity and precision. The blank is determined each day by repeating the entire procedure but excluding the sample. The resulting volume increase is subtracted from subsequent nitrogen measurements.

Water vapor in the nitrogen gas exerts a significant pressure and is most conveniently subtracted from the observed barometric pressure. This partial pressure varies with the temperature of nitrogen and caustic concentration. Several correction tables have been published (25,106,110). Table III(*a*) is practical for most applications.

The barometric pressure is measured to ±0.2 mm. at the time of the analysis. The barometer is mounted in the same room as the combustion apparatus but away from unusual heat sources. Aneroid-type barometers, with built-in temperature compensations, need no corrections as do the glass scale and brass scale mercury barometers (84). However, their accuracy should be checked occasionally against a mercury barometer. Brass scale barometers, the most prevalent type in use, require temperature expansion corrections of the mercury and the brass scale itself, as shown in Table III(*b*) for convenient reference.

2. Calculation

The volume of nitrogen gas that is measured obeys the perfect gas law whereby 1 gram-mole of nitrogen (28.014 g.) occupies 22.414 liters at 760.0 mm. pressure and 273°K. or, simply, 1 cc. of nitrogen under these conditions weighs 1.250 mg. For all practical purposes, the nitrogen volume is not measured under this ideal pressure–temperature condition. Therefore, it must be corrected accordingly.

$$\frac{WT}{PV} = \frac{W_1T_1}{P_1V_1}$$

thus

$$W = \frac{W_1T_1}{P_1V_1} \times \frac{PV}{T}$$

W_1 = 1.250 mg. of nitrogen
T_1 = 273°K. (0°C.)
P_1 = 760.0 mm.
V_1 = 1.000 cc.
V = nitrogen volume after blank subtraction
P = barometric pressure after scale and water vapor corrections
T = 273°K. + t, where t is temperature of nitrogen in degrees centigrade
W = weight of nitrogen to be calculated

The calculation thus becomes

$$W = \frac{(1.250)\ (273.0)}{(760.0)(1.000)} \times \frac{(P)(V)}{273.0 + t} = \text{mg. of nitrogen}$$

Since per cent nitrogen = [nitrogen (mg.)]/[sample (mg.)] × 100, the combined calculations, with the standard temperature–pressure–weight numbers combined, appears as

$$\text{Per cent nitrogen} = \frac{(P)\ (V)}{273.0 + t} \times \frac{44.899}{\text{mg. of sample}}$$

G. EVALUATION OF DUMAS METHODS

Present knowledge and apparatus applied to the theories of Dumas have achieved a remarkable degree of precision; 0.04 to 0.2% standard deviations have been reported. The speed of analysis has been shortened considerably so that five to six routine analyses an hour is common practice. The method is further enhanced by the simplicity of operation now possible. The operator is no longer required to check for "microbubbles" in the nitrometer before and after each analysis; prior knowledge of nitrogen content is less critical; slow combustion of samples is impractical; and the so-called "caustic drainage" delays of 20 to 30 minutes are eliminated.

However, the simplicity in operation is attainable only with a thorough understanding of the procedure's requirements and limitations and with painstaking care in assembling the apparatus. Failure to consider all the ramifications will result only in perplexing and exasperating difficulties.

By removing the subjective skills, a considerable degree of confidence and reliability in the method has developed during the past few years. Formerly an exclusive research service, it is gaining in acceptance for control and production applications as the result of automation and commercialization. The compilation of diverse applications in Table I is a strong indication of this acceptance.

However, there also have been failures where the problems of sampling heterogeneous materials below the gram range have not been resolved. Until scaling-up of the micro procedures to accommodate gram samples is made practical, this conflict will continue to exist. A careful evaluation of sampling must be given full consideration, for many apparently heterogeneous samples have been successfully analyzed on the milligram range, as indicated by the natural products and biological specimens listed in Table I.

1. Applications

Exploitation and extension of the method's recent developments have reached far beyond the previous limited applications of pure organic compounds. Table I indicates to what extent the method has now been used. Sampling is the major problem. Special attention is drawn to the modifications of sample preparation in Section II-A and to the removal of interferences from samples in Section II-B-3. The analysis of trace quantities, particularly in parts per million, has been successful in some applications, but the problem of large organic samples leaves much to be desired. When mixed with inorganic materials or aqueous suspensions, the resulting large samples are not difficult to handle. Some inorganic materials have been successfully analyzed with the addition of vanadium pentoxide or an organic accelerator, such as benzoic acid, for certain nitrates.

2. Space and Operator Requirements

The apparatus is mounted on a bench of normal height (*ca.* 36 inches) that has adequate electrical services, usually of 110 v. a.c. and 15 to 20 amp. overload protection. If burners are used for sample furnaces, then gas services are necessary. The length of bench space needed for the assembly varies from $1^1/_2$ feet for an automatic vertical combustion system to 5 feet for less compact arrangements. A convenient out-of-the-way place near the apparatus is needed for the carbon dioxide tank. Short tanks can be stored in a large cabinet under the bench if such a space is available. Additional bench space adjoining the apparatus should be provided as a work area where reagents and combustion tubes can be packed and set aside. A 2 to 3 foot space is usually adequate, depending on the length of the combustion tubes in use. Special precautions must be taken in locating the nitrometer to avoid exposing it to heat or air temperature fluctuations. The sample furnaces are mounted for easy movements, and the combustion tube, posttreatment tube, and absorption chamber are securely mounted for easy removal. The rest of the system is rigidly secured, preferably to a common sturdy platform. Stopcocks and joints are shielded or located away from unusual heat sources that soften lubricants and destroy their seal.

Conventional micro and ultramicrobalances sensitive to vibrations, heat, and humidity require special installations away from the nitrogen apparatus, usually in a separate balance room. Manufacturer's specifications and recommendations are to be consulted. Torsion and electromagnetic balances (e.g., Cahn Electrobalance, Cahn Instrument Co., Paramount, California) with appropriate microgram sensitivity but insensitive to the

interferences mentioned above, are most conveniently located on the bench beside the apparatus. This close proximity reduces operator fatigue and increases efficiency and productivity. By observing the combustion procedure while weighing samples, the operator is able to spot deviations from normal combustions and apply suitable corrections as experience dictates.

The requirements of an operator are those for an average technician of sufficient manual dexterity to weigh small samples on a sensitive balance, pack combustion tubes, manipulate the valves and furnaces through simple movements, and make visual observations of the nitrogen volume, its temperature, and the barometric pressure. Also a knowledge of basic arithmetic involving multiplication and division is needed for calculating the per cent nitrogen.

However, an experienced analyst, thoroughly familiar with the procedure's requirements and limitations, must be called upon to assemble and check the apparatus before it is turned over to a technician. Automatic apparatus, preassembled by the manufacturer, demands less technical skills to operate but requires still greater attention of the experienced analyst to the instrumental components for obtaining the maximum precision and service possible. For preventive maintenance and repair of automatic instruments, the manufacturer's operating manual (27) should be consulted.

3. Diagnosis and Correction of Failures

Difficulties revolve around four faults: leaks in the system, contamination of carbon dioxide, exhaustion of reagents, and improper combustion. Occasionally difficulties arise because of an oversight of just one of the many strict requirements. The more frequent failures, how they manifest themselves, and corrective techniques are presented in Table IV as a quick reference to the requirements detailed in Sections II and III.

4. Instrumentation

Until recently, no instrumentation of significance had been developed. Aside from making compact unitized voltage controls and slow drive mechanisms for the forward movement of a sample burner, the subjective skills of the operator were required for manual manipulations and controls. Many laboratory suppliers have such combustion stands for supporting a combustion tube and two furnaces. Furnaces capable of maintaining 900°C. are suitable for Dumas combustion analysis. Adaptation of this type of equipment to the individual's existing facilities necessitates the additional fabrication of glassware and supports.

TABLE IV. Failures, Diagnoses, and Corrections

Symptom	Correction
A. High Blank Volume	
a. High blanks indicate impure carbon dioxide.	Purge or replace carbon dioxide supply.
b. Variable blanks indicate	
(*1*) contamination from grease, plastic, rubber, etc.	Clean or remove source of contamination.
(*2*) post heater at wrong temperature.	Readjust post heater to required temperature.
(*3*) air leak between combustion tube and nitrometer.	Locate and eliminate leak. Plunger inserts of electric solenoid valves occasionally fail and require replacement.
c. Blanks increasing in volume indicate	
(*1*) caustic absorption solution nearly exhausted.	Recharge absorption chamber with fresh potassium hydroxide solution.
(*2*) posttreatment reagents exhausted.	Repack posttreatment tube.
d. Initially high blanks that diminish with each test indicate contamination from new reagents or glass tubes.	Preignite reagents, combustion tube, and posttreatment tube. Preheat packed posttreatment tube at 700°C. for 15–30 min. if necessary.
B. Low Nitrogen Values	
a. Incomplete combustion of sample from	
(*1*) premature degradation.	Chill sample area of the combustion tube during preheat period.
(*2*) refractory materials.	Add vanadium pentoxide–copper acetate–cobalt oxide oxidation catalysts and, if necessary, lengthen combustion time and increase furnace temperature.
b. Gas leak in either the combustion assembly or measuring chamber.	Locate and eliminate leaks.
c. Exhausted copper packing which allows nitrogen oxides to pass into nitrometer as caustic soluble gases.	Replace packing in posttreatment tube.
d. Nitrogen bubbles sticking to stirring bar or mercury surface.	Dislodge all bubbles by rapid agitation of bar or manipulation of small wire across mercury surface with a magnet.
C. Shrinking Nitrogen Volume	
a. Carbon dioxide in measuring chamber as a result of	
(*1*) exhausted potassium hydroxide.	Recharge absorption chamber with fresh potassium hydroxide solution and flush measuring chamber with nitrogen.
(*2*) inadequate scrubbing of bubbles in absorption chamber.	Check for proper agitation and gas flow. Also, with Pregl nitrometer, check for proper dilution of combustion products with carbon dioxide in the Zimmerman reservoir. Flush measuring chamber with nitrogen before attempting subsequent analyses.

TABLE IV (*continued*)

Symptom	Correction
c. Slightly soluble combustion products in measuring chamber usually from materials containing sulfur or halogen.	Flush measuring chamber with nitrogen and add vanadium pentoxide oxidation catalyst to subsequent samples containing sulfur. For interference from halogen, extend copper oxide packing of the combustion tube 40–50 mm. beyond furnace area to provide cool condensing surface for halide complexes.
D. High Nitrogen Volume	
a. Incomplete combustion of sample from (*1*) premature degradation.	Chill sample area of the combustion tube during preheat period.
(*2*) too rapid pyrolysis of large 20–100 mg. samples with methane or other caustic insoluble gases blown through hot packing too fast for adequate oxidation.	Slow forward movement of furnace to control combustion rate; not to exceed bubbling rate of sweep period. Also add vanadium pentoxide and cobalt oxide oxidation catalysts for additional source of oxygen.
(*3*) release of methane or ethane possibly from materials with high alkoxyl content.	Add cobalt oxide as catalytic agent.
b. Posttreatment reagents exhausted. Refer also to *A. High Blank Volume*, above.	Repack posttreatment tube.
E. Instrumental Difficulties	
a. Inadequate carbon dioxide flow indicated by flowmeter	
(*1*) during purge to atmosphere indicates blocked gas line or exhaustion of carbon dioxide source.	Replenish carbon dioxide source if necessary or clear obstruction.
(*2*) during sweep into nitrometer indicates its capillary inlet is blocked with carbonates.	Remove and clean absorption chamber of carbonate obstruction with dilute acid.
b. Sudden increase or too rapid carbon dioxide flow as shown by flowmeter indicates a leak.	Check source of leak and seal or replace defective parts. Rubber seals for combustion tube require frequent replacement.
c. Mercury or caustic moving back through inlet tube during analysis indicates caustic has migrated back of inlet seal.	Remove and clean inlet with dilute acid.
d. Erratic and sluggish movement of the caustic level in an agitated absorption chamber during volume measurements indicates some caustic has entered the measuring chamber.	Disassemble the measuring chamber, clean thoroughly, dry, and relubricate.

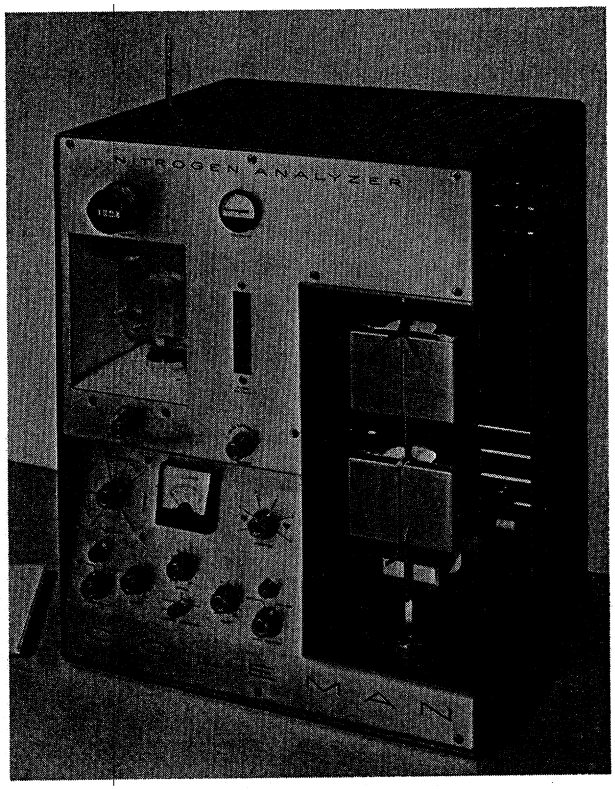

Fig. 6. Automatic nitrogen Dumas analyzer (courtesy Coleman Instrument Co., Maywood, Illinois).

An automatic nitrogen analyzer now has been developed (48). All furnace movements and valve manipulations are electrically synchronized through a timed-programming device. Except for glass combustion tubes and absorption chamber, the entire system is metal. The combustion tube and furnaces are mounted vertically for compactness instead of in the classical horizontal position. This space-saving advantage is not without complications, for an unusual amount of heat rises from the furnaces to overheat the upper mount of the combustion tube. Therefore, a reflective heat shield is secured below this mount. This heat problem also causes premature degradation of liquid or low-melting samples unless they are previously chilled.

The commercial adoption of this apparatus, ass hown in Fig. 6, utilizes components specifically designed for this method to provide the maximum performance available. This preassembled unit relieves the

operator of building his own apparatus from various equipment not always to his choosing. The operator is required only to attach a sample-packed combustion tube to the apparatus, adjust the measuring syringe for the initial volume measurement, and then turn on the automatic cycle switch. Every furnace movement and valve opening is programmed so that the instrument returns all controls to their original starting positions when the cycle is complete. For routine applications, the operator is completely free during the cycle (6 to 8 minutes) to carry out other duties. At the conclusion of the cycle, only the measuring syringe is readjusted to show the increase in volume. As with any automation, diverse applications do not always conform to the existing programming; however, with manual controls provided on the instrument, the various phases of the cycle can be altered to the individual's requirements.

When in regular use, the price of automation has been considered by Hilton (53) to be returned within a year through savings in man-hours. Justification for its use becomes difficult to assess when only sporadic use is anticipated. It has been the author's experience that, with sporadic demands for nitrogen analysis, similar man-hour savings are also obtained because the speed with which the warmup and analyses are completed quickly relieves the operator for other duties.

Modifications to the program of the automatic Coleman Nitrogen Analyzer have been reported (161). Also extensive programming and mechanization have been developed by Dorfman *et al.* (34).

5. Other Developments

Ultramicro determinations of nitrogen using microgram samples (10^{-6} g.) have been developed (49,75,79). More recently, Hozumi and Kirsten (58) devised a sealed capillary for an ultramicro procedure whereby the remaining nitrogen volume was displaced by mercury and the mercury weighed.

Mizukami and Miyahara (108) built an eight-unit multiple combustion system to complete 32 analyses a day. They combusted eight samples simultaneously into eight different nitrometers with a specially designed furnace holding all the combustion tubes. Standard deviations of 0.14% are reported.

Nitrogen measurement by gas chromatography is a new approach to microanalytical combustion methods which has tremendous appeal, since in addition to nitrogen, carbon and hydrogen can also be measured from the combustion of only one weighed sample (55,135). Several novel techniques for separating and measuring the combustion products have been developed. Maresh *et al.* (96a) collected nitrogen, carbon dioxide, and acety-

lene (released by reacting water with calcium carbide) in a silica gel trap cooled with liquid nitrogen. Then the gases were released into a classical gas chromatographic column.

Walisch (175a) developed an instantaneous combustion method and retained the water on silica gel temporarily while the mixture of nitrogen and carbon dioxide passed on through the first cell of the katharometer. An integrator totaled the sum of the two gases. Then carbon dioxide was removed by Ascarite while nitrogen passed through the second cell, which resulted in a negative response on the integrator, with the difference remaining as the carbon dioxide measurement. Then water was released for measurement by heating the silica gel.

Simon *et al.* (154) and Clerc and Simon (26a) used three sets of katharometers in series with a selective absorbent separating the sample and reference cells of each set. All combustion products were first collected in an evacuated chamber to make a homogeneous mixture. The gas mixture was then passed through the evacuated katharometers into a second smaller chamber until the gas flow stopped when pressure between the chambers equilibrated. Thus a static or steady state within the katharometers developed. An imbalance between the sample and reference cells of the first katharometer was a measure of water removed in the absorption column between the cells. Carbon dioxide and nitrogen remained in both cells to cancel their effect on the katharometer. Carbon dioxide was removed in the second katharometer for measurement and, similarly, nitrogen was measured in the third katharometer.

Unfortunately, special electronic instrumentation is needed which is beyond the abilities of any normal laboratory to build. Some instruments, however, are being commercially developed through the joint efforts of several analysts and instrument manufacturers. Whether this new approach can augment or actually replace the established Kjehdahl and Dumas methods cannot be predicted on the basis of the present state of the art. Various factors limiting the accuracy and performance of chromatographic measurements have been discussed by Clerc and Simon (26a). An accuracy for nitrogen of 0.3% has been reported by Clerc and Simon (26a) and Walisch (175a).

III. RECOMMENDED LABORATORY PROCEDURES

Two procedures are described, the first using an agitated absorption chamber, and the second using a Pregl nitrometer and modified Zimmerman mercury reservoir. Although the first procedure is faster by a few minutes and requires fewer manipulations, the second method has the advantage of

using classical equipment already in use in many laboratories. The same degree of sensitivity and precision is obtained by either method.

The commercialized automatic nitrogen analyzer is based on the first method. Specific details of construction, operation, and repair should be elicited from the manufacturer's operating manual (27). Aside from the automated features of the instrument, all techniques and precautions given in the following method must also be observed in using the instrument. Manual controls give the operator complete control for modified combustions needed with problem samples.

Equipment for both methods is available from various commercial supply houses. However, some fabrication in the laboratory is necessary to assemble the second system. For the ultimate in sensitivity and precision, all-glass joints are recommended. This includes ball-and-socket joints on the combustion tubes.

The actual combustion techniques are identical for the two methods. Although a period of time is suggested for each phase of the analysis, the operator must first establish the timing needed to obtain the maximum precision from his own assembly. When Dry Ice in Dewar flasks or acid–bicarbonate reagents in Kipp generators are the only available source of carbon dioxide, the rapid purge and sweep techniques must be modified to accommodate the limited carbon dioxide reserve. However, these sources are not recommended if tank carbon dioxide is available.

A. RAPID COMBUSTION METHOD

1. Apparatus and Reagents

The modified Shelberg two-unit combustion system and agitated absorption chamber shown in Fig. 7 consists of the following items: carbon dioxide tank with two-stage reducing valve, flowmeter, three needle valves, two three-way directional flow valves, combustion tube, two sample furnaces, posttreatment tube, heater, absorption chamber with magnet, magnetic stirring motor, and a measuring syringe. Details of these items and the necessary reagents are described below.

Carbon dioxide tank. A two-stage reducing regulator (see Note *1*) is attached to a prepurified tank of carbon dioxide (e.g. Matheson Co., Coleman Grade CO_2) and air is purged from the regulator (see Note *2*). The low-pressure discharge is then adjusted to 3 to 5 p.s.i. Flexible metal tubing connects the regulator to the flowmeter (see Note *3*).

Flowmeter. The flowmeter, with two floats of different densities, provides an extended flow range between 5 and 300 cc./minute.

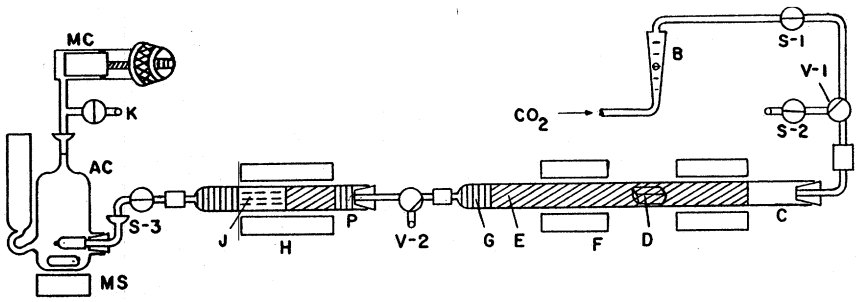

Fig. 7. Rapid combustion apparatus with agitated absorption chamber and digital measuring syringe. *B*, flowmeter; *C*, Vycor or quartz combustion tube; *D*, sample area; *E*, copper oxide packing; *F*, sample furnaces; *G*, glass wool retaining plug; *H*, post-treatment heater; *J*, copper packing; *K*, vent to expel nitrogen; *P*, post treatment tube; *S-1*, *S-2*, *S-3*, precision flow controls; *V-1*, *V-2*, directional valves; *AC*, agitated absorption chamber with slitted rubber inlet valve and magnetic bar; *MC*, measuring chamber with precision, syringe connected to digital counter by micrometer screw; *MS*, magnetic stirring motor.

Precision flow controls. Three needle valves or precision flow control type stopcocks, designated *S-1*, *S-2*, and *S-3*, are mounted in the positions indicated in Fig. 7. Valve *S-1* is adjusted to a flow of 200 to 300 cc./minute, *S-2* a flow of 5 cc./minute (see Note *4*), and *S-3* a flow of 20 to 30 cc./minute.

Three-way stopcocks. Two 120° three-way stopcocks designated as *V-1* and *V-2* have a 2-mm. bore in the plug and 2-mm. capillary side arms (see Note *5*). Valve *V-1* directs the carbon dioxide to either the combustior tube or the atmosphere through *S-2*. Valve *V-2* connects the combustion tube to the atmosphere or, during the analysis, to the posttreatment tube. No flow adjustments are made with these directional stopcocks.

Combustion tube. The quartz or Vycor combustion tube has a 9-mm. bore, 1 to 1.5-mm. wall thickness, and a 400 to 525-mm. length (see Note *6*). A one-hole neoprene stopper (see Note *5*) connects the tube to the carbon dioxide supply. A 25 to 50-mm. plug of glass wool is packed into the exit end of the combustion tube (see Note *7*); the tube end is attached to valve *V-2*. To facilitate packing the sample boat in the proper position every time, a fire-resistant reference mark is painted or etched on the tube to indicate the midpoint between the two sample furnaces when they are 75 to 100 mm. apart during the preheat period.

Furnaces. Two 75 to 100-mm. long electric furnaces (see Note *8*) that open from the side are mounted on a stand which also supports the combustion tube. They are movable both along the length of and away from the tube. Their temperature must maintain the combustion tube at 850 ±

25°C. Their lengthwise movements are independent of each other. The first furnace, preceding the sample, moves across the sample area against the second furnace for the initial combustion. Then both move simultaneously an additional 25 to 50 mm. toward the posttreatment tube.

Posttreatment tube. The Pyrex or Vycor tube of 10 to 12-mm. O.D. has sufficient length to extend 50 to 75 mm. beyond its heater. Equal amounts of preignited copper and copper oxide (see Sections II-C-2 and II-C-4) are densely packed within the area of the heater and retained by glass wool plugs.

Heater. An electric heater, maintaining the posttreatment tube at 500 ± 50°C. (see Note *9*), is securely mounted.

Absorption chamber. The agitated absorption chamber (see Fig. 2), containing a plastic-covered magnet, is filled with 50% potassium hydroxide solution through the rigid side arm to the capillary reference mark *R*. Then it is attached to the apparatus as shown in Figs. 4 and 7 and centered over a magnetic stirring motor.

Measuring syringe. The metal measuring syringe (see Fig. 3(*b*)) is connected to the needle valve *K* for venting, and the absorption chamber *AC* through suitable $^1/_{16}$- to $^1/_8$-inch stainless steel tubing and metal ball-joint (see Notes *3* and *10*). The syringe is moved into its cavity by turning the knurled knob of the digital counter to a reading of 0.2 to 0.3 cc. The vent is temporarily opened to expel excess nitrogen. Caustic forces the excess nitrogen out as it seeks its original level in the capillary.

2. Procedure

Assembly and preparation of the train. Before assembling any components, the analyst is cautioned to review carefully Section II-B and remove all interferences his equipment may contain. The success and simplicity of operation depends entirely upon the serious attention given to the cleaning and assembling of each item.

The two-stage regulator, sealed to the pretreated tank of carbon dioxide with a graphite-impregnated washer, is purged of air before being attached to the flowmeter. Purging is accomplished by raising the delivery pressure to 40 to 60 p.s.i., turning off the tank valve, and venting the regulator to the atmosphere. This is repeated four to six times. The delivery pressure is then readjusted to 3 to 5 p.s.i. for normal operation. The tank is secured near the assembly but away from the furnaces.

The entire assembly is mounted on a common rigid platform in a straight line, as indicated in Fig. 7. All components are securely clamped. The combustion tube is densely packed with CuO to 60 to 80 mm. from the

open end and secured in a manner to permit easy removal. The posttreatment tube is also densely packed with copper and copper oxide and secured. The system is then checked for leaks by increasing the delivery pressure to 10 p.s.i., closing stopcock *S-3*, turning valves *V-1* and *V-2* to connect the carbon dioxide supply to the posttreatment tube, and, when the system is at full pressure, stopcock *S-1* is closed for a 5-minute period. When stopcock *S-1* is quickly reopened, there must be no discernible movement of the float in the flowmeter. The delivery pressure is then reduced back to 3 to 5 p.s.i. and stopcock *S-1* readjusted to a flow of 200 to 300 cc./minute.

Checking for contaminants. The system is checked next for contaminants after the following preparations. Air in the combustion tube and posttreatment tube is purged to the atmosphere through stopcock *S-3* after temporarily disconnecting the absorption chamber. The carbon dioxide flow is then stopped by *S-3*, the absorption chamber reconnected (see Note *3*), the measuring vent *K* closed, caustic in the capillary pushed below the reference mark with the syringe (see Note *10*), and the magnetic stirring motor turned to high speed. Stopcock *S-3* is then carefully opened to allow a very slow flow (5 to 10 cc./minute) of carbon dioxide into the absorption chamber as air trapped in the chamber's inlet is purged into the absorption chamber. The carbon dioxide flow is finally increased to 20 to 30 cc./minute with *S-3* and valve *V-1* is turned to vent the carbon dioxide to the atmosphere through *S-2*. When bubbling in the absorption chamber stops, the stirrer is turned off; bubbles adhering to the magnet are dislodged by full-speed agitation; and, after all bubbles have risen to the capillary, the caustic level is raised to the reference line. The digital counter is read and the syringe temperature recorded. Any contaminants in the combustion system are now allowed to accumulate for the next 10 minutes, after which the stirrer is turned on high speed, and valve *V-1* again turned to pass carbon dioxide into the absorption chamber for 2 minutes. Valves *V-1* and *V-2* are then returned to "standby" as shown in Fig. 7, and the stirrer is stopped. Any measurable volume increase (0.001 to 0.002 cc.) after a correction for any temperature change (see Table II) indicates contamination that must be traced to its source and eliminated.

With the system satisfactorily passing the leak and contamination tests just described, subsequent checks are made only when unexplainable difficulties occur during blank analysis tests.

A final check is now made for contaminants from heated reagents and glassware. The posttreatment tube is first heated to its operating temperature (*ca.* 500°C.). Expanding carbon dioxide bubbling into the absorption chamber is so slow that agitation is unnecessary at this time. The sample furnaces, backed away from the combustion tube, are heated to

825 to 850°C. The "method of combustion" described below is then carried out without any sample so that any sizeable volume increase (0.02 to 0.10 cc.) indicates the presence of contaminants which must be burned off the reagents. In this event, the posttreatment tube is heated at a much higher temperature (700 to 750°C.) for 15 to 30 minutes with a slow flow of carbon dioxide purging the tube. When the heater returns to its normal temperature, the check is repeated. A blank of 0.004 to 0.008 cc. is ideal; however, larger blanks (0.010 to 0.020 cc.) that are reproducible can be tolerated when trace measurements are not being made (see Note *11*).

Blank determination. The system's blank of caustic-insoluble gases from unaccountable sources is determined before starting a series of analyses. Usually only 0.004 to 0.008 cc. in size and reproducible to ±0.001 cc. (see Note *12*), blanks are measured by repeating every step in the "method of combustion" below without any sample. Any oxidation aids used with samples must also be used in the "blank" analysis to check for interferences. The blank value is then subtracted from the observed nitrogen volume of every subsequent analysis.

Sample preparation. Samples are weighed on a microbalance with due regard to the special handling techniques usually employed for hygroscopic, volatile, static, or other weighing interferences. Their size varies inversely with nitrogen content, 2- to 10-mg. samples for nitrogen above 1%, 10- to 20-mg. samples for levels of 0.1 to 1.0%, and 20 to 100 mg. for trace measurements below 0.1% (see Note *13*). A reference compound of known purity is first analyzed at the beginning of each series to verify the precision expected from the apparatus. Since each laboratory is primarily involved with sample structures peculiar only to its industry or service, reference compounds are best chosen from representative structures frequently encountered.

Nonvolatile liquids can be weighed in boats containing preignited copper oxide powder or other inert material that will keep the liquid from migrating up the sides of the boat. Otherwise, melting point capillaries cut to 20 to 30-mm. lengths serve as excellent containers. Volatile samples can be weighed in similar capillaries having one end sealed and the other constricted to allow a fine (24 to 26 gage) hypodermic needle to enter. A static charge sometimes accumulates at the opening of a combustion tube that can cause loss of some samples. Materials affected in this manner can be smothered with copper oxide powder to hold them in the boat during introduction into the combustion tube. Materials requiring oxidation-catalytic aids (see Section II-A) are also smothered with the necessary metal oxides. The addition of both vanadium pentoxide and cobalt oxide to every sample is suggested when a variety of structures are encountered,

especially when sulfur is known to be present. When they are being added, caution must be exercised not to knock any sample from the boat.

3. Method of Combustion

After the furnaces have reached operating temperature, or following a delay in excess of 10 minutes from a preceding analysis, the entire combustion system is purged with carbon dioxide. Valve *V-1* is turned to purge carbon dioxide through the combustion tube to the atmosphere through valve *V-2*. After a 1- to 2-minute purge, the magnetic stirrer is turned to high speed and *V-2* is turned (see Note *14*) to pass carbon dioxide into the absorption chamber for 1 to 2 minutes. Both valves are then returned to their "standby" positions, *V-1* connecting carbon dioxide to the atmosphere through *S-2*, and *V-2* connecting the combustion tube to the atmosphere. Henceforth these positions will be referred as "standby." The stirrer is turned off and any bubbles sticking to the magnet are dislodged.

A combustion tube is packed to the reference mark with copper oxide. Tapping is necessary to assure a dense packing (see Note *15*). With the combustion tube held securely in a horizontal position, the sample boat is carefully set inside and pushed against the packing (see Note *16*). Additional copper oxide is then added to the tube within 3 inches of the end and the packing is tapped again. The sample-packed tube is then attached to the combustion assembly and *V-1* is turned to purge air from the tube. During the 45 to 60-second purge period (see Note *17*), the caustic level in the absorption chamber is adjusted to the reference line by the measuring syringe. The digital number on the counter is recorded (see Note *18*) along with the air temperature adjoining the syringe. The stirrer is turned on, valve *V-2* is turned to the posttreatment tube, and *V-1* is returned to "standby." Both sample furnaces are brought forward to surround the combustion tube but 40 to 50 mm. away from the reference mark (see Note *19*). After this preheat period of 2 to 3 minutes (see Note *20*), the furnace nearest *V-1* is moved quickly (see Note *21*) over the sample area without uncovering the preheated area entirely. If the furnaces do not touch, the second one is pushed back against the first. When bubbling in the absorption chamber diminishes, usually in 2 to 3 minutes, both furnaces are advanced toward the posttreatment tube about 25 to 50 mm. for a final combustion period of 2 to 3 minutes (see Note *22*). If bubbling persists, this period is continued until the bubbles stop.

Next, all combustion products are swept into the absorption chamber with carbon dioxide by turning valve *V-1*. During this 90 to 120-second sweep period (see Note *17*), the sample furnaces are backed away from the

combustion tube and, if necessary, a reflective shield set in front of them. The carbon dioxide flow of 20 to 30 cc./minute fluctuates, so readjustment of valve *S-3* may be required.

Finally, the sweep is concluded by returning both *V-1* and *V-2* to "stand-by" and, after bubbling stops, the magnetic stirrer is stopped. Sticking bubbles are dislodged from the magnet with full-speed agitation. The dropped caustic level is returned to the reference mark (see Note *23*) by withdrawing the measuring syringe. The changed digital numbers are recorded along with the syringe temperature and the barometric pressure. The next analysis is immediately started (see Note *24*) by attaching the next sample-packed combustion tube and repeating the procedure without the preliminary steps described first.

4. Calculations

The analytical data and corrections are numerous and, therefore, recorded in an orderly manner to avoid confusion and erroneous calculations. The corrected data consists of: sample weight (W), volume of nitrogen (V), nitrogen temperature (t), and barometric pressure (P) (see Section II-F).

Refer to Tables II and III for the corrections to be subtracted from the nitrogen volume and barometric pressure (see Note *25*). In addition to these corrections, the "blank determination" described above is subtracted from the nitrogen volume.

The calculation appears, with the corrected data, as:

$$\text{Per cent nitrogen} = \frac{P \times V \times 44.899}{W \times (273.0 + t)}$$

5. Notes

1. Only two-stage reduction valves can maintain uniform pressures at such a low working level of 3 to 5 p.s.i. This is essential to have consistent and reproducible flows of carbon dioxide.

2. Purging may have to be repeated if the tank is not used for several days (see Sections II-B-1 and II-C-1).

3. Metal tubing connections, pipe threads, needle valves, stopcocks, and ball and socket joints are made "bubble" tight at 10 p.s.i. with Grade T Apiezon grease (see Section II-C-6).

4. Contaminants originating from the valve packing of the tank are not allowed to accumulate when carbon dioxide is not passing through the combustion system. They are continuously purged to the atmosphere through valve *S-2*.

5. Clamped ball and socket joints are preferable; however, heavy-walled neoprene tubing is acceptable.

6. Length of the combustion tube varies with the available furnaces and supports. At least 120 to 150 mm. of the tube ends extend beyond the heated area.

7. The glass wool plug should be at least 40 to 50 mm. from the furnace area to leave a cool zone of copper oxide packing where halide interferences can condense (see Section II-B-1-b).

8. Gas burners capable of maintaining 800 to 900°C. may be used if electric furnaces are not available. Special care must be taken to isolate the nitrometer from excessive heat discharged by them.

9. Before the heater is turned on, the posttreatment tube is purged of air with carbon dioxide.

10. The mercury-filled measuring capillary (see Fig. 60.3(*a*)) can be used instead, but its smaller capacity and buret-type scale make it a second choice. Its lower fabrication costs may appeal to the occasional user, for there is no loss in precision with proper estimation of the mercury level between scale divisions. Also, the volume of buffer air it contains is so small that no temperature corrections are needed between the initial and final volume measurements.

11. Reagents giving high blanks must be reignited. New combustion tubes, used for the first time, will give a higher blank. Therefore, an analysis must not be attempted in a new tube until the tube has been preignited by a blank analysis.

12. Reproducible blanks of 0.010 to 0.020 cc. are acceptable if the sensitivity and precision are not critical. However, additional blank checks are made after four to eight analyses to be sure it has not changed. The sources of this interference must be located and removed (see Section II-B).

13. Because of decomposition problems, certain materials require oxidation-catalytic aids. Refer to Sections II-A and II-B and to Table I.

14. Special precaution must be exercised in turning valve *V-2*. It must never connect the hot posttreatment tube to the atmosphere.

15. An electric vibrating tool facilitates packing the copper oxide and avoids accidentally breaking the combustion tube from excessive tapping.

16. Unless the sample boat contains oxidation-catalytic aids with the sample, it can be spilled at the reference mark to increase its dispersion in the packing.

17. The purge and sweep periods need to be extended for large combustion systems. Each system must be checked for the time required with a 25% increase in time added as a safety factor.

18. Nitrogen remaining in the syringe from a previous analysis does not have to be vented at this time if the syringe's capacity will not be exceeded. The final volume–temperature readings for the preceding analysis serve as the initial readings for this analysis.

19. Premature degradation or loss of sample must be prevented. Liquids and solids that are heat sensitive may require chilling of the sample area during the purge and preheat periods. In the event of extensive chilling with Dry Ice, the preheat period is extended several minutes after chilling has stopped to minimize thermal shock in the tube.

20. The length of time for preheat depends entirely upon the heat reserve in the furnaces. Refractory insulated furnaces heat much faster than radiant furnaces having reflective shells.

21. Samples heavier than 20 mg. are burned slowly by moving the furnace forward by increments such that evolved combustion products bubbling into the absorption chamber do not exceed the rate of bubbling observed visually during the sweep period.

22. To facilitate heating the space between the furnaces, a final combustion period is carried out by pushing the sample furnace over this area.

23. Caustic in the capillary is invariably trapped by nitrogen gas below. This must be pushed down from the capillary before the caustic level is raised to the reference line. A simple inward movement of the measuring syringe quickly drops the trapped liquid below the nitrogen gas.

24. In the event of a delay in excess of 10 minutes before starting the next analysis, an accumulation of contaminants may occur that must be purged from the system. The preliminary steps described first must be repeated.

25. Temperature-compensated aneroid barometers do not require a scale expansion correction.

B. PREGL NITROMETER—ZIMMERMAN RESERVOIR METHOD

1. Apparatus and Reagents

The Pregl nitrometer is used with a modified Zimmerman reservoir and the modified Shelberg two-unit combustion system shown in Fig. 8. The combustion assembly, techniques, and reagents are identical to those in the rapid combination method. However, the additional manipulations required for sweeping combustion products into the nitrometer make this procedure a few minutes slower.

Because of the general availability of Pregl nitrometers, this method may be more applicable in many installations. Most important, the same high

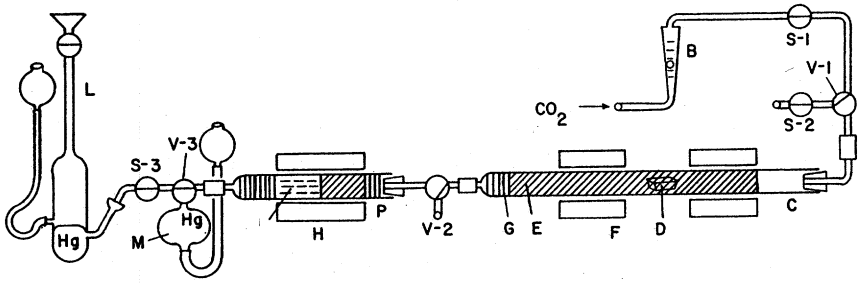

Fig. 8. Combustion apparatus with Pregl nitrometer and Zimmerman reservoir. *B*, flowmeter; *C*, Vycor or quartz combustion tube; *D*, sample area; *E*, copper oxide packing; *F*, sample furnaces; *G*, glass wool retaining plug; *H*, posttreatment heater; *J*, copper packing; *L*, Pregl nitrometer; *M*, Zimmerman reservoir filled with mercury; *P*, posttreatment tube; *S-1*, *S-2*, *S-3*, precision flow valves; *V-1*, *V-2*, *V-3*, directional valves.

degree of sensitivity and precision is achieved with both methods. The Apparatus and Reagents and Procedure below are identical to those in the rapid combination method (Section III-A) except for the reservoir and nitrometer.

Modified Zimmerman reservoir (see Fig. 8). The sturdy 80 to 100-ml. capacity glass reservoir has a 2 mm. I.D. capillary outlet at the top and a 5 mm. I.D. outlet at the base. The base is attached to a leveling bulb of similar capacity through clean heavy rubber tubing that will not bulge or burst from the pressure of mercury. The reservoir is rigidly clamped to a substantial support. The leveling bulb is mounted to a similarly rugged support such that it can be easily raised and lowered. The capillary outlet of the reservoir, having a standard socket joint, is clamped to the three-way stopcock *V-3*. Apiezon grease must be used to seal the joint and lubricate the stopcock. With the base of the leveling bulb raised almost to the height of the stopcock, clean mercury is added until the reservoir is filled to the stopcock plug. A 40 to 50-cc. reference level is marked on the reservoir to indicate when all combustion products have been swept from the combustion assembly (see Note *1*).

Pregl nitrometer. The Pregl nitrometer, shown in Fig. 1, has a 1.5-cc. nitrogen capacity and is connected through clean rubber tubing to a leveling bulb. The tubing, previously leached with caustic solution to remove talc and other sediment, is secured to the glassware with wire or tubing clamps. Without clamping, the extremely slippery caustic-wetted tubing will come off. Sufficient mercury is added to the nitrometer to cover the capillary inlet 5 to 10 mm. For dislodging bubbles adhering to the mercury surface, a steel wire, approximately 10 to 15 mm. long, is also added. It is moved

around by holding a magnet near the nitrometer. Sufficient potassium hydroxide (50%) is then added through the leveling bulb to completely fill the nitrometer and half of the leveling bulb. Do not allow caustic to touch the stopcock; it is kept below at the upper scale graduations (see Note *2*). The leveling bulb is secured to an adjustable clamp so that it can be raised to the height of the nitrometer scale. The capillary inlet is attached to stopcock *V-3* through the flow control *S-3*. Valve *S-3* is adjusted for a flow of 20 to 30 cc./minute from the carbon dioxide-filled reservoir *M* when the mercury-filled leveling bulb is elevated. This flow is, at best, estimated by observing the same bubble rate of carbon dioxide when flowmeter *B* indicates a 20 to 30 cc./minute flow.

Refer to Section III-A-1 for the remaining apparatus and reagents.

2. Procedure

Assembly and preparation. Refer to *Assembly and preparation* of the rapid combustion method (Section III-A) for the carbon dioxide supply and combustion assembly. The mercury reservoir is attached to valve *V-3*. This valve is located between the posttreatment tube and flow control *S-3* (see Fig. 8). Air in the mercury reservoir is expelled by raising the mercury-filled leveling bulb until mercury touches the plug of *V-3*. The valve is then turned to connect the posttreatment tube to valve *S-3*. This position will hereafter be referred to as "standby."

The assembled apparatus is then checked for contaminants as described in Section III-A. The mercury reservoir is not used during these tests.

Blank determination. As described in Section III-A.

Sample preparation. As described in Section III-A.

3. Method of Combustion

The techniques of adjusting the system before analyses, packing and attaching the combustion tube, and purging air from the system with carbon dioxide are described in Section III-A. At the conclusion of the purge, valve *V-2* is turned to connect the combustion and posttreatment tubes (see Note *3*), *V-1* is turned to stand-by, and *V-3* is turned to connect the reservoir to the posttreatment tube. The mercury-filled leveling bulb is lowered to the 40 to 50 cc. reference mark on the reservoir. The combustion tube is now preheated by bringing both sample furnaces over the combustion tube 80 to 100 mm. apart, with the sample area midway between (see Note *4*). After 2 to 3 minutes of preheating, the furnace near valve *V-1* is moved forward over the sample area (see Note *5*), keeping some of the preheated area within the furnace. If the furnaces do not touch, the

second one is moved back against the first. The rate of combustion is indicated by the dropping mercury level in the reservoir. After the initial combustion of 2 to 3 minutes, the cool area between the furnaces is heated by advancing both furnaces together 25 to 50 mm. toward valve *V-2*. This final combustion period is continued 2 to 3 minutes after the last visible change in the mercury level is noted. All combustion products are now swept into the reservoir with carbon dioxide by turning *V-1* to the combustion tube to give a carbon dioxide flow of 20 to 30 cc./minute. The furnaces are moved back away from the combustion tube during this sweep period. Valve *V-1* is returned to stand-by when mercury drops to the reference mark on the reservoir. Before transferring gases to the nitrometer, the caustic leveling bulb is raised to the upper scale divisions and excess nitrogen is vented through the stopcock above. A small buffer zone of nitrogen is left between the stopcock and the scale below. The caustic leveling bulb is held level with the meniscus in the measuring capillary and the meniscus level is recorded (see Note *6*).

Stopcock *V-3* is now turned to connect the reservoir to the nitrometer. The mercury-filled leveling bulb is raised and valve *S-3* is adjusted to transfer the gases within 90 to 120 seconds at a flow of 20 to 30 cc./minute (see Note *7*). When mercury enters the plug of *V-3* (see Note *8*), the stopcock is returned to stand-by and carbon dioxide is swept into the nitrometer by opening *V-1* to the combustion tube. The microbubbles suddenly appear within a few seconds and the sweep is concluded by returning *V-1* and *V-2* to stand-by. Nitrogen bubbles stuck to the mercury in the nitrometer are dislodged with the wire and magnet. The caustic leveling bulb is releveled to the lowered meniscus. The change in volume in the nitrometer, the air temperature adjoining the nitrometer, and the barometric pressure are all recorded. If necessary, the barometer temperature is also noted (see Note *9*).

The next analysis is now started (see Notes *7* and *10*).

4. Calculations

As described in Section III-A, except that no correction is necessary for buffer air temperature changes (see Note *6*).

5. Notes

1. When combusting samples larger than 10 mg., the total volume of combustion products and carbon dioxide is increased beyond the reference mark in the mercury reservoir accordingly.

2. Filling the stopcock and funnel on top of the nitrometer with caustic invariably results in seepage of caustic down into the measuring capillary

above the collected nitrogen. This complication is easily circumvented by never raising the caustic level as high as the stopcock, keeping the meniscus at the uppermost scale divisions. This necessitates an initial reading before nitrogen is swept into the nitrometer. The leveling bulb is raised to this level before the initial reading is observed.

3. As Note *14* in Section III-A-5.

4. As Note *19* in Section III-A-5.

5. As Note *20* in Section III-A-5.

6. The small volume of buffer air, measured before collecting nitrogen in the nitrometer, does not change significantly with temperature variations, so no temperature correction is necessary with the initial measurement.

7. The next sample-packed combustion tube can be attached during this transfer of gases from the reservoir into the nitrometer. But first, valve *V-2* must be returned to "stand-by." The purge is carried out and, following the sweep of carbon dioxide into the nitrometer for the analysis in progress, the next analysis can be continued. This simple expedient reduces the time of analysis by 4 to 6 minutes.

8. Excessive mercury trapped in the plug is pushed into the nitrometer by carbon dioxide. Valve *S-3* must be carefully opened further to allow the mercury to pass through. This problem can be avoided by pinching the rubber tubing to the mercury reservoir so as to expel only the gases without catching mercury in the plug. No gases may be allowed to remain below the plug.

9. As Note *25* in Section III-A-5.

10. As Note *24* in Section III-A-5.

Part 2

Kjeldahl Method. By Clyde L. Ogg

I. INTRODUCTION

Approximately 80 years ago the Kjeldahl method came into being when Kjeldahl (76) first digested organic nitrogen-containing material with sulfuric acid and found the nitrogen to be quantitatively converted to ammonia. Since that time so many modifications of the method have been described and redescribed that to try to link an author's name with that of Kjeldahl to denote all different modifications is impractical. Two names, however, could reasonably be coupled to Kjeldahl's to give due credit to those who contributed most to the development of the method as it is com-

monly used today. Those are Wilfarth (177), who in 1885 first used a catalyst (HgO) to speed the digestion, and Gunning (46), who in 1889 first added potassium sulfate to increase the digestion temperature and thus increase the digestion rate. One of the best historical reviews of the Kjeldahl method was made by Vickery (175), who recommended the adoption of the name Kjeldahl-Wilfarth-Gunning method to describe the present method, and this was adopted by the Association of Official Agricultural Chemists in the 7th edition of their *Official Methods of Analysis* in 1950. In this chapter the single name, Kjeldahl, will be used to denote the commonly used modifications which include the use of potassium sulfate and a catalyst in the sulfuric acid digestion mixture.

Although Pilch (131) was the first to perform Kjeldahl analyses on the micro scale, the commonly used terms macro and micro will be used to designate the scale of the analysis. These terms are more or less arbitrary because there is no well-defined dividing line between them. Those analyses in which digestion flasks of 300 ml. and larger and macro distillation and titration equipment are used will be considered to be macro, and those using micro equipment will be classed as micro, irrespective of the size of sample. Most of the discussion will be centered on these two scales because these are the ones most in use. Regardless of the scale the digestion of the sample is the same. The composition of the digestion mixture and the temperature and time required are essentially the same for both macro- and microanalysis.

There have been a number of good reviews of the Kjeldahl method which, at the time they were written, brought the reader up to date with the developments in the method. Some of the more recent reviews were by Schöniger (146), Kainz (62), Bradstreet (17,18), Kirk (71), and Macdonald (93), the last being specific for the determination of nitrogen in coal and coke. All texts on quantitative organic analysis, such as those by Steyermark (165), Niederl and Niederl (111), Clark (26), Milton and Waters (107), Kirk (72), Roth (133), and Grant (132), discuss and present methods for the Kjeldahl determination. *Standard Methods of Chemical Analysis*, edited by Furman (149), should also be included in this list. *Official Methods of Analysis of the Association of Official Agricultural Chemists* is probably the best reference work for keeping up to date on developments in the method for two reasons; first, both macro and micro methods are subjected to frequent collaborative testing to evaluate new modifications, and second, the book is revised every five years. Collaborative testing with statistical analysis of the results is a most valuable tool in evaluating methods, and applications of this technique to the Kjeldahl method has done much to help arrive at optimum conditions for nitrogen analysis.

The Kjeldahl method is one of the most widely used of all analytical methods, being of prime importance in fertilizer, animal feed, and food analysis. Whenever protein content is an important factor, it has been, and still is, the method of choice to estimate the amount of protein present. The word estimate is used because the protein content is obtained by multiplying the total nitrogen found by the Kjeldahl method by the factor 6.25, unless it is known that for a particular material some other factor is more accurate. The Kjeldahl method measures not only the nitrogen from the protein but also any nitrogen present in other classes of compounds, so the total nitrogen times a factor gives only an estimate of the protein present. Nevertheless, it is frequently the best estimate readily available.

Many industrial processes depend on the Kjeldahl method for quality control; it is used in coal, coke, and oil analysis and in establishing the identity and purity of naturally occurring and synthetic organic compounds containing nitrogen.

A. SAMPLE STRUCTURE PROBLEMS

The Kjeldahl method is not universally applicable. Certain types of compounds require modifications in the method for its successful use. A discussion follows of the limitations of the method as related to the way the nitrogen is linked in the compound.

1. Heterocyclic Nitrogen Compounds

As was stated above, the greatest use of the method has been in the determination of protein nitrogen. Even here, some of the modifications used have produced results that are too low because the digestion temperature was too low or the time too short to completely convert the nitrogen in some amino acids (principally tryptophan which contains the guanidino group) to ammonia. Most Kjeldahl analyses for protein nitrogen, however, have been reasonably satisfactory.

Until recent years many analysts considered that nitrogen present as C—N—C in a ring was too refractory for the Kjeldahl method and could at best be only partially converted to ammonia in the Kjeldahl digestion. This is not so. As long ago as 1920, Phelps (129) showed that nicotinic acid, quinoline derivatives, pyroles, purines, etc., could be analyzed by the Kjeldahl method, provided mercury was used as catalyst, enough potassium sulfate was added to produce a high temperature, and the digestion was long enough. Shirley and Becker (153) confirmed this in 1945, and since then Kjeldahl analysis of materials with heterocyclic nitrogen has

become common. In fact, one of the primary standards currently used for evaluating new modifications is nicotinic acid. There is no question that most heterocyclic compounds require an active catalyst such as mercury and high digestion temperatures to complete the digestion in a reasonable time. Lake *et al.* (82) found that with mercury as catalyst, a temperature of 370°C. and a time of one hour were required for complete digestion of heterocyclic compounds. Steyermark (165) reported that in the analysis of certain condensed pyrimidine and pyrazine ring compounds and in materials with a number of *N*-methyl groups the Kjeldahl method gives better results than the Dumas method.

2. Compounds with N—O Linkages

When the nitrogen in organic compounds is linked to oxygen it is usually necessary to reduce the nitrogen prior to the usual digestion with the sulfuric acid and catalyst. Many different reducing systems have been proposed, but none has proven universally applicable. Reduction with hydriodic acid and red phosphorus as proposed by Friedrich and co-workers (42) has been used successfully by many analysts, whereas others have not been satisfied with this method and have devised other reduction procedures. These have fallen into two general categories, namely, reduction with a metal such as iron or zinc in an acid media, or reduction with carbon, which is usually generated by adding an easily carbonized substance to the sulfuric acid. If the nitrogen is present as a nitrate, other modifications including salicylic acid plus sodium thiosulfate or thiosalicylic acid have been used to pretreat the sample; however, these are coming under scrutiny and other reduction procedures are being tested by the Association of Official Agricultural Chemists. Steyermark (165) has shown that in some compounds with N—O linkage the bond is so weak that a split takes place during the first part of the digestion and no pretreatment is necessary.

3. Compounds with N—N Linkages

Compounds with N—N linkage are even more difficult to analyze than materials with N—O linkages. With these, reduction before digestion is imperative, and it is unfortunate that there is no method which is universally applicable. Azo compounds, in general, are more easily analyzed than hydrazo and the latter more easily analyzed than 1,2-diazines, 1,2,3-triazoles, etc. No Kjeldahl method has been reported which is satisfactory for the analysis of triazoles.

The various reduction procedures for N—O and N—N will be discussed later in some detail. Of the many that have been devised, none can be en-

dorsed with confidence. Collaborative test of several of these methods (116,119,181,182) have shown that some analysts can obtain good results with one method, others with another modification, but with no method so far tested have all analysts obtained satisfactory results for compounds with N—O and N—N linkages. The fact that some analysts obtain good results on a given compound with a specific method, whereas others do not, indicates that the limits of the variables in the method are not sufficiently well defined.

B. APPARATUS

Two units are required for the Kjeldahl determination, one the digestion and the other the distillation apparatus. Both multiple-unit digestion and distillation apparatus are commonly used on the macro scale. Although multiple-unit digestion apparatus is in general use on the micro scale, the distillation apparatus used usually permits only one distillation at a time.

1. Micro Digestion Apparatus

Most micro digestion apparatus commercially available today are six- or 12-unit electrically heated digestion racks as shown in Figs. 9 or 10. The six-unit model, Fig. 9, developed by the Arthur H. Thomas Company,* has individual heater control, which permits heat output control to each flask. This is of increasing importance because newer methods are specifying the heat applied to each flask in terms of the time required to bring a specified volume of water from 25°C. to boiling. American Society for Testing Materials standard for Kjeldahl digestion racks (2) specifies that the heaters must raise 15 ml. of water in a 30-ml. digestion flask from 25°C. to boiling in not less than 2 nor more than 3 minutes. This is easily attained with heaters having individual controls and usually not too difficult with racks in which all units are controlled with a single variable transformer. With gas-heated racks this control may be more difficult. The 12-unit rack, Fig. 10, developed by the American Instrument Company, can either be operated in two units of six with a 5-amp. variable transformer for each set of six, or as a 12-unit rack with a 10-amp. variable transformer controlling all 12 heaters. The advantage of the 12-unit model is that it requires less bench space.

These digestion racks are usually designed for 10- or 30-ml. Kjeldahl flasks, but modified racks which will accommodate 100-ml. flasks are also available. The 10- and 30-ml. flasks, including the Soltys flask which is used when spattering is a severe problem, have been standardized by the

* Mention of company and trade names does not imply endorsement by the United States Department of Agriculture over others not named.

ASTM (2). Specifications for 100-ml. flasks were not included because these are not normally used in microanalysis. However, they are often useful when analyzing materials such as plant extracts which are extremely low in nitrogen.

Other means of supplying the heat required to digest the samples have been used. The most common of these is a sand bath, which may be acceptable in certain instances but is not recommended for general use. An aluminum block with 12 drilled holes 1 inch I.D. by 2 inches deep and containing a 200-w. electric heater was recommended by Evans *et al.* (37).

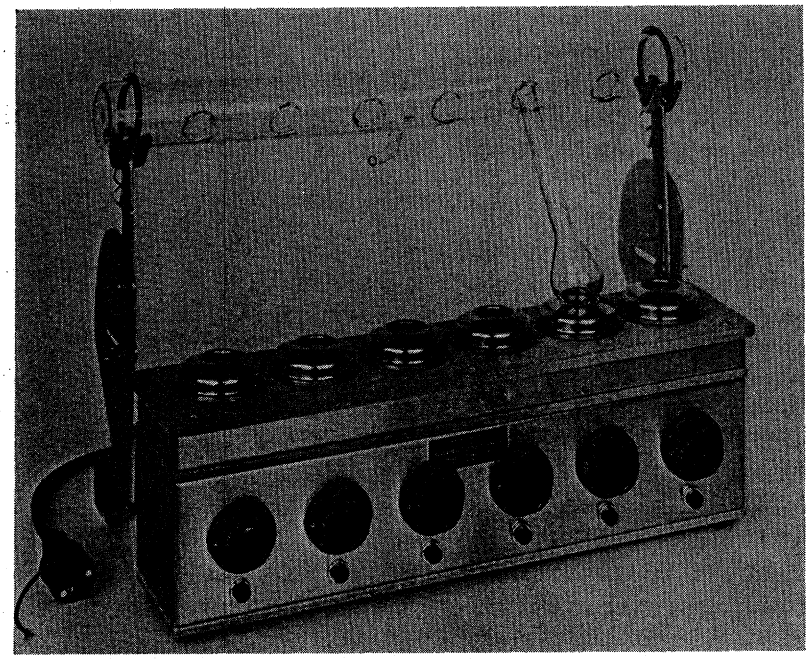

Fig. 9. Six-unit micro Kjeldahl digestion rack with individual heater control.

This unit would provide the necessary temperature control, but tubes rather than flasks have to be used for sample digestion. Tubes should be satisfactory, provided foaming, bumping, and spattering are not excessive during digestion.

Acid fumes formed during the digestion are mostly sulfur dioxide with smaller amounts of sulfur trioxide, and sometimes halogen acids. These must not be vented to the room. The commercial digestion units are provided with glass fume ducts which may be connected to water aspirators to remove acid fumes. The fume duct for the 12-unit circular digestion rack

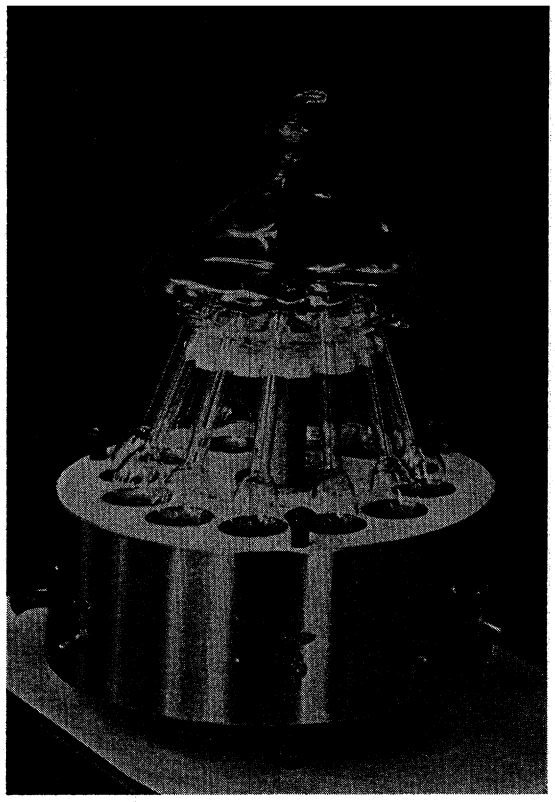

Fig. 10. Twelve-unit rotary micro Kjeldahl digestion rack.

shown in Fig. 10 requires a high capacity aspirator. If a high capacity aspirator is not practical because of low water pressure or small water lines, a laboratory vacuum line may be used, provided it is equipped with an efficient scrubber and trap.

2. Micro Distillation Apparatus

Distillation of the ammonia in microanalysis is performed by steam, preferably using all-glass apparatus. Numerous stills have been designed and used by various analysts and two of these have been recommended by the American Chemical Society Committee on Microchemical Apparatus (166) and standardized by the ASTM (2). The still preferred by many is that shown in Fig. 11, the so-called one-piece apparatus. The still plus its steam generator (a resin kettle and cover coupled with an immersion heater)

make a compact, easily operated, and easily cleaned unit. The still is attached to the center opening of the cover and the heater is fastened in two of the side openings, leaving the third opening available for replenishing the water in the still. The rate of steam generation is controlled by regulating the output of the heater with a variable transformer, and steam

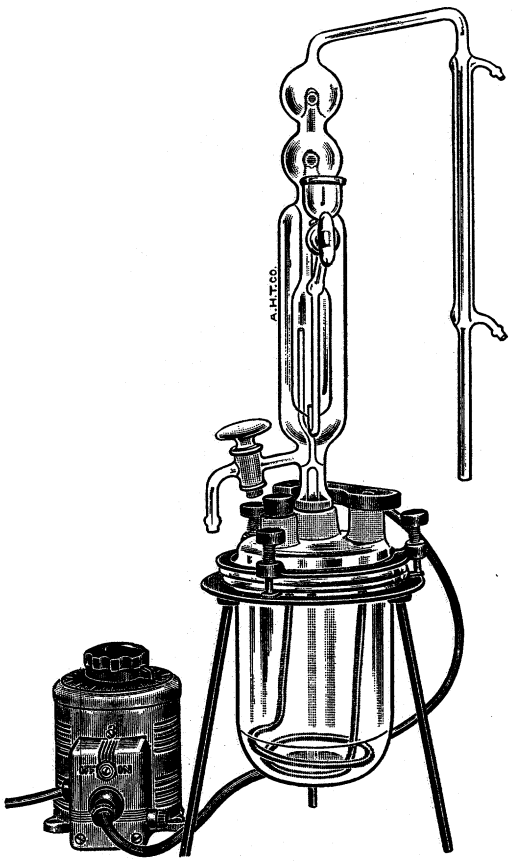

Fig. 11. One-piece micro Kjeldahl distillation apparatus.

generation can be stopped almost immediately by turning off the current. The still is cleaned by placing a beaker of water under the tip of the condenser; condensation of steam in the still and head space creates a partial vacuum which draws water vigorously through the still and cleans it for the next distillation.

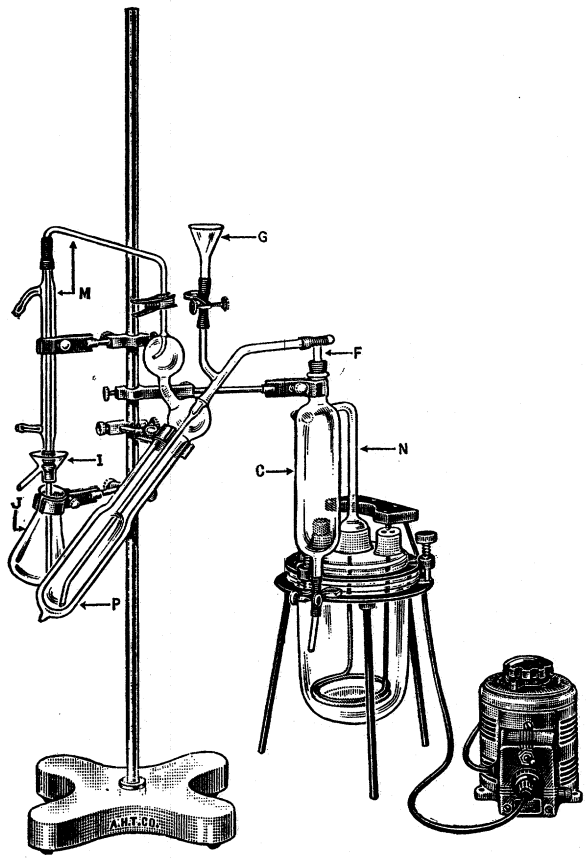

Fig. 12. Modified Parnas-Wagner micro Kjeldahl distillation apparatus.

The second apparatus standardized by the ASTM (2), Fig. 12, is a slightly modified Parnas-Wagner (123) still. This apparatus has been used for many years with satisfactory results. Its operation is similar to the one-piece still but it is not as compact and not as rugged. The rate of distillation with both apparatus is limited by the efficiency of the condenser. For this reason the specifications for both call for West condensers rather than the Liebig type formerly used.

When using either of the above apparatus, the digest must be transferred quantitatively from the Kjeldahl flask to the still. Some stills have been designed to eliminate this step by attaching the flask to the still head by a standard taper joint. An available commercial model of this type is supplied by the American Instrument Company as a two-piece head.

Another distillation apparatus which does not require transfer of the digest was described by Asami (3). This system has the advantage of eliminating the necessity of transferring the digest, but it has two disadvantages, namely, the flasks are more expensive and condensate buildup during distillation will be greater because the flask is not protected by a jacket as in the one-piece or Parnas-Wagner apparatus.

Simultaneous multiple distillations in micro Kjeldahl determinations are rarely if ever performed because the time required for a distillation is so short. There are times, however, when a multiple unit would speed the distillation and titration process, particularly if transfer of the digests was not required. The relatively large number of methods reported in recent years which are designed to avoid the distillation process indicate a need for more rapid, multiple-unit distillation apparatus.

Other stills worthy of mention are those of Kirk (70), Marino (97), and Schöniger and Haack (147), all one-piece apparatus. Scandrett (141) designed a still which used superheated steam from a steam line rather than a steam generator. This steam source would probably be satisfactory in some laboratories and not in others; even in those laboratories where it appeared suitable, frequent blank analyses would seem to be advisable.

A number of years ago silver condenser tubes were considered necessary in microanalysis. This was shown by an AOAC collaborative study (180) to be incorrect. Results obtained with all-glass condensers were equally as good as, if not better than, those obtained with silver condenser tubes, indicating that borosilicate glass condensers are satisfactory.

3. Macro Digestion Apparatus

When more than an occasional analysis is required, the digestion racks usually used have six, or a multiple of six, units. These are commonly supplied with individually controlled electric heaters. This feature is becoming more desirable as the importance of controlled heating of the digest is being realized. The present AOAC method (103) specifies that the heater be adjusted to cause 250 ml. of water in a 500 to 800-ml. flask to come to a rolling boil in approximately 5 minutes. Although gas-heated digestion racks are used, controlling the heat output within fairly close limits is difficult. Fume control on the macro scale is a greater problem than on the micro scale. Lead fume ducts are used with either a specially designed, oversized water aspirator or, if the fumes can be vented to the outside air, an exhaust fan.

4. Macro Distillation Apparatus

Distillation apparatus, like the digestion racks, usually have six, or a multiple of six, units. Direct rather than steam distillation is used exclusively, whereas almost the converse is true for micro methods. Again, either gas or electric heat is used, with electric being preferred, but temperature control is not as important as in the digestion step. Block tin condenser tubes sealed in copper condenser jackets are commonly used. Because direct distillation is used and because granular zinc (which is added to the distillation flask) generates hydrogen gas, creating an alkaline spray, the efficiency of the distillation trap is of major importance. If the rate of distillation is fast there is danger of mechanical carryover of the basic solution leading to erroneously high values. Scrubber-type traps such as those of Davidson or John (179) reduce this source of error and permit more rapid distillation rates.

Perrin (128) found that when copper sulfate was used as a catalyst, alkali carryover was increased unless the copper was precipitated as the sulfide. Paul and Berry (126) noted that unless an efficient trap was used, the alkali carryover amounted to 0.1 to 0.2% protein per gram of sample. Obviously, the amount of alkali carryover is related to the rate of distillation, the amount of excess alkali in the solution, the surface area of the zinc, and the size of the flask. Since the effect of these various factors is not known quantitatively, the analyst must use an efficient trap and not distil too rapidly.

C. SAMPLE DIGESTION

It must be remembered that anything said of the digestion on either the micro or macro scale is equally true for the other, with the possible exception of the use of oxidants such as H_2O_2 in micro digestions. The reaction rates, catalysts, and temperature effects are not changed by reducing the scale of the digestion. There is evidence, however, that the ratio of the area of the glass–liquid interface to the volume of digest affects the digestion rate. It is known that superheating occurs at the glass surface; so the increase in digestion rate, which apparently occurs with higher ratios of interfacial area to volume of digest, may merely be a temperature affect.

The digestion step, i.e., the quantitative conversion of all nitrogen in the sample to ammonia, is by far the most difficult part of the analysis. Determining the optimum conditions and the best catalyst have been the objectives of most of the studies conducted and the subject of most of the controversies about the method. In the digestion step the nitrogen is reduced as the organic matter is oxidized, leading to carbon dioxide, water, and

ammonia as end products, with the ammonia being retained in the acid digest, provided the proper conditions are maintained.

As long as the nitrogen is linked to carbon and hydrogen, or carbon only as in heterocyclic compounds, no serious problems are encountered in the digestion, provided the proper catalyst and temperature of digestion are used. When the nitrogen is linked to carbon and oxygen, or carbon and another nitrogen, the method usually must be modified by the addition of some reagent to aid in the reduction of the nitrogen to ammonia.

The sample digestion will be discussed under five headings: temperature effect, catalysts, reducing agents, oxidizing agents, and closed-tube digestion. The first two, temperature and catalysts, are of primary importance; the third, reducing agents, applies only to those compounds with N—O and N—N linkages; while the fourth, oxidizing agents, must be mentioned because numerous workers have tried to speed the digestion by their use. The use of oxidizing agents, however, is questionable in the light of present knowledge of the method. Closed-tube digestion is a means of obtaining high digestion temperatures without the addition of a salt.

1. Temperature Effect

Gunning (46) in 1889 was the first to report the use of potassium sulfate to raise the temperature of the digest and shorten the time required. The effect of varying the salt/sulfuric acid ratio (g./ml.) was reported by Phelps (130) in 1921. His work showed that with mercuric oxide as catalyst and a 2:3 salt/acid ratio, pyridine salts could be analyzed with a $2^1/_2$-hour digestion. As the amount of catalyst was decreased the ratio had to be increased if the digestion time was constant. Shedd (150) related boiling temperatures to salt/acid ratio as long ago as 1927, but unfortunately he used sodium instead of potassium sulfate and this work was not followed up for over 20 years. He used 18 g. sodium sulfate to 25 ml. acid with 0.7 g. Hg and obtained clearing in 10 minutes. With a total boiling time of 20 minutes his results compared favorably with those obtained after 3 to 5 hours digestion using less salt. Harrel and Lanning (50) reported similar observations with sodium sulfate in 1929, and Lepper (86) in 1930 found that rapid digestion was obtained if the potassium sulfate/acid ratio was 3:4. In spite of these observations, the highest ratio commonly used until after 1950 was in the AOAC Kjeldahl-Wilfarth-Gunning method (114) (then called the Kjeldahl-Gunning-Arnold method), which called for 15 to 18 g. potassium sulfate to 25 ml. of acid. In 1950, Ogg and Willits (118) measured the boiling temperatures of potassium sulfate–sulfuric acid solutions, with ratios varying from a low of 1:4 to a high of 7:8, and found the boiling points to increase from 332 to 358°C. The digestion rates roughly

followed the general rule that a temperature increase of 10°C. doubles the reaction rate. This led to an improved micro method by which refractory heterocyclic compounds could be analyzed but required a 3-hour digestion. The following year, Lake *et al.* (82), in a collaborative study on the determination of nitrogen in petroleum oils, extended the study of the effect of temperature and determined the upper limits for the temperature when potassium sulfate is used to raise the boiling point of the mixture. They determined that if the temperature exceeded 410°C., nitrogen loss may occur. With a temperature of 360°, 2 hours were required for a complete digestion, whereas at 370° only 1 hour was needed thus verifying the work of Ogg and Willits. To avoid the possibility of exceeding 410°, Lake and co-workers recommend adjusting the amount of potassium added to give a temperature of 370°. This amount will vary with atmospheric pressure or elevation.

Bradstreet (20) made a rather thorough study of the effect of salt/acid ratio on nitrogen recovery. He used the term "acid index," which he defined as the milliliters of acid divided by the grams of potassium sulfate. He concluded that the limiting acid index at the end of the digestion was 0.88 (1.144: salt/acid ratio) and that lower indices would likely lead to nitrogen loss. He determined the amount of acid lost by boiling plus that lost by reaction with different sample materials. For known materials the acid consumed during digestion can be approximated from the equation for the oxidation of the material with sulfuric acid, or it can be determined by weighing the charged flask before and after digestion and subtracting the weight of the sample from the difference. The weight of the ammonia in the digest is ignored, as the acid loss need only be known approximately so that a safe salt/acid ratio will not be exceeded at the end of digestion. When using high salt/acid ratios, as many of the newer methods do to minimize the digestion time, it is essential to consider the amount of acid used to oxidize the sample, otherwise nitrogen losses may occur because of too high digestion temperatures at the end of the digestion. As a guide to the amount of acid lost during digestion, Kirk (71) estimated that 1 g. of carbohydrate requires 7.3 g. sulfuric acid or approximately 4 ml., while 1 g. of fat requires 17.8 g. or almost 10 ml. of acid. Consideration of the acid loss is usually more critical in macro- than in microanalysis because the sample/acid ratio is usually lower in the latter; however, it cannot be ignored in either.

Some of the recently described proceaures which make use of the high salt/acid ratio are the AOAC official micro method (115,116) which specifies a 0.95:1 acid/salt ratio, Perrin's macro method (128) in which the ratio at the start is 0.8:1, McKenzie and Wallace's (98) micro method which calls

for a 1:1 ratio of sodium sulfate to acid, and a very recent method by Baker (7) in which the ratio is raised to 1.5:1, considerably above the safe limit set by Bradstreet. These methods require digestion times from approximately 20 minutes to 1 hour for complete digestion, depending on the nature of the material being analyzed.

The most rapid and reliable methods will use a salt/acid ratio which will result in a maximum boiling temperature of 370 to 380°C. The importance of the heat output specifications and control of the digestion rack heaters discussed under apparatus now becomes apparent. Using the proper salt/acid ratio would be of little value if there were not enough heat available to bring the digest to its true boiling point. Too much heat applied to the digestion flasks can be deleterious, particularly when high salt/acid ratios are used, because superheating normally occurs at the glass–liquid interface. This superheating may amount to several degrees and can lead to nitrogen loss from digests which are already near the critical limit of approximately 400°C.

Salts other than potassium or sodium sulfate have been used to increase the boiling temperature of the digest. Gerritz and St. John (44) recommended the use of a mixture of 64% dipotassium hydrogen phosphate and 36% sodium or potassium sulfate. Stubblefield and DeTurk (170) tested the same two salts plus ferric sulfate and concluded that a mixture of 10 parts of dipotassium hydrogen phosphate and 6 parts of ferric sulfate caused most rapid digestion. Unfortunately, these studies were made before the temperature effect was fully appreciated and the boiling temperatures of various digestion mixtures were not measured. Perrin (128) completely replaced potassium sulfate with dipotassium hydrogen phosphate and obtained an increase in digestion rate and temperature but noted flask etching and an occasional low result, possibly from too high a temperature. Further study of the use of phosphate salts relating salt concentration, digest boiling point, digestion rate, and nitrogen recovery would permit better comparison of the effect of sulfate and phosphate salts and might lead to some interesting results. It should be pointed out that Kjeldahl and also Wilfarth (177), who initiated the use of mercury catalyst, both used a mixture of sulfuric acid and phosphorus pentoxide.

2. Catalysts

The most controversial subject related to the Kjeldahl method has been the choice of catalyst. Since Wilfarth (177) first proposed the use of mercuric oxide in 1885, about 40 elements have been tested for catalytic activity. Although Osborn and Wilkie (122) tested 39 elements and found the order of efficiency of the best ten to be Hg, Se, Te, Ti, Mo, Fe, Cu, V, W,

TABLE V
References to Various Catalysts Used or Tested in the Kjeldahl Method

Catalyst	Selected references
Mercury	7, 31, 38, 61, 103, 117, 120, 121, 122, 125, 126, 128, 129, 139, 140, 148, 153, 177, 178
Selenium	7, 31, 38, 85, 117, 121, 122, 125, 139, 140, 148, 178
Copper	7, 31, 61, 117, 122, 124, 125, 126, 128, 139, 140, 153
Iron	7, 122
Platinum	7, 122, 164, 172
Molybdenum	7, 122
Vanadium	7, 122, 124, 129
Chromium	7, 122
Tellurium	7, 122
Others	122, 129
	Mixed Catalysts
Mercury plus selenium	12, 29, 31, 103, 117, 120, 125, 129, 139, 153, 178
Mercury plus copper	29, 31, 148
Copper plus selenium	29, 31, 117
	Catalysts Causing N Loss
Selenium	29, 31, 38, 109, 120, 121, 125, 139, 140, 178
Platinum	164, 172

and Ag, most of the studies have been on Hg, Se, and Cu or mixtures of these three. Table V presents a list of some of the elements studied and some selected references. An attempt will be made to summarize the findings of the numerous authors who have studied the three most popular catalysts, Hg, Se, and Cu. Although the results are often contradictory, the mass of evidence gives a rather clear picture.

Mercury. It has been well established that Hg or HgO is the best catalyst for the Kjeldahl digestion. Mercuric oxide is usually preferred because it is more easily handled than mercury. In studies like those of Osborn and Wilkie (122), Baker (7), and Phelps (129), in which a number of catalysts are compared, mercury is almost invariably listed as the best single catalyst. Schwab and Schwab-Agallidis (148) found that mercury gave a true catalytic effect, i.e., the reaction velocity depended on the concentration of mercury in the digest and not on sample/mercury ratio. Reaction velocity as related to mercury concentration over the temperature range of 255 to 280°C. (about 100° below the recommended temperature range) was expressed by

$$k_{Hg} = \frac{\alpha[Hg] + \beta[Hg]^2}{1 - b[Hg]}$$

which indicates that mercury influences the reaction in two ways simultaneously, one involving one mercury ion, and the other, two. The temperature dependence of the two constants was expressed by

$$\log \alpha = 11.49 - 26{,}200/4.57T$$

$$\log \beta = 9.49 - 18{,}000/4.57T$$

The superiority of mercury as catalyst is most apparent if heterocyclic nitrogen compounds are analyzed when comparing catalysts. Most authors who claim to be able to analyze these materials use mercury catalyst. Even though Phelps (129) had demonstrated in 1920 that quantitative results could be obtained for heterocyclic compounds if mercury was used as a catalyst, a collaborative study of micro methods conducted in 1949 resulted in only four of 12 laboratories obtaining good analyses for nicotinic acid and all four used mercury as catalyst. When the study was repeated and all collaborators used a specified procedure requiring mercury catalyst, all obtained satisfactory results. Shirley and Becker (153) also came to the conclusion that mercury must be present for satisfactory results with heterocyclic nitrogen compounds. Even for easily digested materials such as proteins, AOAC (61) studies have shown that the results with mercury are higher than those with copper as catalyst.

Selenium. The second best catalyst, selenium, was proposed by Lauro (85) and has been the subject of much controversy, chiefly because of the nitrogen loss that may occur if the temperature of the digest is too high or the time too long.

Of the 39 elements tested by Osborn and Wilkie (122), selenium was rated second to mercury and this has become generally accepted. If clearing time alone were considered, selenium would probably rank above mercury. Schwab and Schwab-Agallidis (148) found that the reaction rate with selenium catalyst was very rapid initially, followed by a gradual logarithmic decay. They postulated that selenous acid is the active form, being reduced to selenium by the organic matter more rapidly than the selenium is reoxidized to selenous acid.

Selenium, either as the metal, selenite salt, or selenium oxychloride, is seldom used alone. It is usually used with mercury for the purpose of increasing the digestion rate over that with mercury alone. Davis and Wise (31) compared the digestion rates for mercury, selenium, and copper, singly and in all combinations and over a wide range of salt concentrations, and found the rate for mercury plus selenium to be faster than for mercury alone for some materials but not for others. Shirley and Becker (153) observed that selenium increased the rate of digestion of nicotinic acid but

not of pyridine. This probably explains why some authors (103,117,121, 139) have claimed that addition of selenium increases the digestion rate, while others (7,122) claimed no increase over that with mercury alone. In the analysis of nicotinic acid, Willits *et al.* (178) found that amounts of selenium sufficient to exert a catalytic effect in the presence of mercury also caused nitrogen loss.

It is well established that the presence of selenium in the digest may lead to loss of nitrogen (see Table V). Davis and Wise (31) showed that with mercury plus selenium catalysts the greatest loss of nitrogen occurred at the highest salt concentration or temperature. Willits *et al.* (178) confirmed this and showed that the higher the selenium content the greater the loss, and that presence of mercury increased the loss caused by the selenium. Patel and Sreenivasan (125), on the other hand, found that the presence of mercury reduced the probability of loss due to selenium. The following reactions were proposed by Sreenivasan and Sadasivan (158) to explain the action of selenium:

In sulfuric acid:

$$Se \rightarrow SeO_3^{2-} \leftarrow SeO_4^{2-}$$

In sulfuric acid plus mercury salt:

$$Se \rightarrow SeO_3^{2-} \rightarrow SeO_4^{2-}$$

In sulfuric acid plus mercury salt plus organic matter:

$$Se \rightarrow SeO_3^{2-} \rightleftharpoons SeO_4^{2-}$$

If these reactions are correct, mercury might be expected to increase the nitrogen loss caused by selenium as Willits and his co-workers reported.

Several points can be made concerning the use of selenium in the Kjeldahl digestion: (*1*) Its catalytic effect is second only to mercury; (*2*) it can cause loss of nitrogen if too much selenium is used, the temperature is too high, or the digestion too long; (*3*) when used with mercury, particularly at high digestion temperatures, it is as likely to be detrimental as beneficial; and (*4*) therefore the digestion rate should be increased by raising the temperature rather than by adding selenium.

Copper. Although copper has been used for years as a catalyst in Kjeldahl digestions, there is conclusive evidence that it is not as good as mercury. Osborn and Wilkie (122) rated copper as the seventh most effective catalyst behind Hg, Se, Te, Ti, Mo, and Fe. Paul and Berry (126) found that the digestion time required with copper was twice that for mercury. Schwab and Schwab-Agallidis (148) were not able to detect any perceptible catalytic effect of copper on the oxidation of aniline.

The mass of evidence, however, indicates that it does catalyze the oxidation of many materials.

The proponents of copper as a catalyst have usually favored it because, unlike mercury, it does not complex with ammonia in alkaline solution and therefore need not be precipitated from solution before distilling the ammonia. This advantage is offset by the following disadvantages: (*1*) A collaborative study (61) has shown that even with proteinaceous materials mercury gives better precision and higher results; (*2*) copper is not a suitable catalyst for heterocyclic nitrogen compounds (117,153); and (*3*) the presence of unprecipitated copper in the solution during distillation tends to increase the amount of mechanical carryover of the alkaline solution in the macro determination wherein mossy zinc is used (128). Because of these findings, the AOAC no longer includes copper as a recommended or optional catalyst in its official methods.

Platinum. Of the elements tested for catalytic effect, the only one found to interfere in the determination was platinum. Ulsch (172) reported that platinum caused low results and others have verified this. Steyermark (164) found that the presence of a platinum boat in a micro digestion could lead to complete loss of the nitrogen present.

3. Oxidizing Agents

The reason for the use of oxidizing agents is the same as that for catalysts and salts, i.e., to speed the digestion. The agents commonly used have been hydrogen peroxide, potassium permanganate, and perchloric acid. Usually they have been used only in micro methods. Of the three, hydrogen peroxide has usually been the oxidant of choice, but the advisability of using even as mild a reagent as this is questionable. It must be kept in mind that the nitrogen must be quantitatively reduced to ammonia while the rest of the organic material is being oxidized.

Although Koch and McMeekin (77) recommended using hydrogen peroxide, Miller and Miller (104) pointed out that proper addition of the oxidant is important and that it must not be added until after the sample has been partially digested. Sreenivasan (157) observed that hydrogen peroxide may cause low nitrogen if added in large amounts. When using the high salt/acid ratio of 1:1, McKenzie and Wallace (98) did not observe any benefits from added hydrogen peroxide.

Perchloric acid was recommended as a digestion aid by Pepkowitz and Shive (127), the oxidant being added after the digest had cleared. Bradstreet (18) found that perchloric acid could cause nitrogen loss, but concluded that, if it could be added in the right amount at the correct time and temperature, its use might be beneficial. Kaye and Weiner (68) observed

that the use of perchloric acid with heterocyclic compounds caused nitrogen loss. The other common oxidant recommended by Beet (9) is potassium permanganate. In his method the permanganate may act as catalyst as well as oxidant since no salt or catalyst is added. The permanganate must be added at intervals, however, until the digest clears and the solution is then brought just to the boiling point. On the other hand, Paul and Berry (126) found no advantage from adding permanganate to conventional digests and stated that it may cause low results.

The use of oxidants may be summarized as follows: (*1*) Addition of an oxidant such as hydrogen peroxide, perchloric acid, or potassium permanganate can decrease the digestion time; (*2*) unless the oxidants are added in small amounts under the proper conditions their use may lead to nitrogen loss; and (*3*) if mercury and a high digestion temperature are used, the disadvantages from using oxidants outweigh any advantages.

4. Reducing Agents

Compounds containing N—O or N—N linkages usually must be pretreated with a reducing agent or subjected to reducing conditions to reduce the nitrogen if connected to oxygen, or to split the N—N bond before completion of the digestion. Numerous agents and techniques have been tried but no universally satisfactory procedure has been developed.

The more classical procedure is that of Friedrich *et al.* (42), which uses hydriodic acid and red phosphorus as reducing agents. If the sample is volatile, reduction is carried out in a sealed tube; if not volatile, it s refluxed with hydriodic acid. The acid and the iodine formed in the process are distilled and then the normal Kjeldahl digestion carried out. Many compounds with N—O and some with N—N linkage may be analyzed by this procedure, but collaborative studies of the method using methyl orange (181), acetone-2,4-dinitrophenyl hydrazone (119), and 1,2,3-benzotriazole (119) showed that some analysts obtained good results for the first two compounds whereas others did not. No one recovered all the nitrogen from the benzotriazole.

In another study (182), sodium hyposulfite ($Na_2S_2O_4$) was the reducing agent, and nicotine picrate and *p*-nitrochlorobenzene the test samples. Seven of the 14 collaborators obtained good results for nicotine picrate whereas the values reported by the other half were low and erratic. For the *p*-nitrochlorobenzene, only 5 of 13 collaborators obtained good results. The same five also analyzed the nicotine picrate satisfactorily.

Another method for reducing the nitrogen has been the use of such metals as zinc, iron, and aluminum in acid media. Dickinson (32) pro-

posed a macro procedure and Steyermark *et al.* (169) a micro method making use of zinc and formic or acetic acid, followed by iron and hydrochloric acid to effect the reduction of the nitrogen in N—O and N—N linkages. Ma *et al.* (91) described a similar procedure for aromatic nitro compounds but omitted the addition of iron. One objection to these methods is that the sample must be soluble in organic acid–ethanol solution. Esafov (36) developed four methods depending on the type of compound, and in two of these zinc was added to a mixture of the sample, sulfuric acid, and potassium acid sulfate. For nitro and azo compounds, the mixture was maintained at 120–130°C. for 1 hour prior to the digestion. Heterocyclic azo compounds with one nitrogen in the ring were heated with zinc for 2 hours at 100°, 2 hours at 150°, and 1 hour at 250° before digestion. The amount of potassium acid sulfate was increased fourfold when heterocyclic nitrogen was present. Steyermark's micro modification of Dickinson's method was subjected to collaborative testing using acetone-2,4-dinitrophenylhydrazone and *p*-nitrochlorobenzene. The results for *p*-nitrochlorobenzene were better than those for the phenylhydrazone, but again only half of the collaborators' means were within ±0.2% of theoretical. Since all collaborators received the same samples and identical directions, it is apparent that the possible variables in the method were not sufficiently well defined.

The third general category of reduction methods makes use of systems in which the sample is heated with carbon at comparatively low temperatures for an extended period prior to digestion. In these methods, compounds are added which carbonize readily and liberate sulfur dioxide slowly as the carbon is oxidized at low heat. These are commonly referred to as carbon reduction methods, but the role of the carbon is not clear; i.e., does the carbon or the sulfur dioxide reduce the nitrogen or are both required? Bradstreet (19,21) has made extensive studies of this type of reduction procedure and recommends the use of sucrose. Esafov (36) described two methods using glucose, one for hydrazones and the other for heterocyclic azo compounds with two nitrogens in the ring. In both Bradstreet's and Esafov's methods the reducing mixture is maintained at a specified temperature for a fixed time before the final digestion. Bradstreet's macro procedure for the reduction of the nitrogen calls for 0.1 to 0.2 g. sample, 0.5 g. sucrose, and 25 ml. of sulfuric acid maintained at 90–100°C. for 1 hour, while the micro methods of Esafov specify 10 to 20 mg. sample, 0.25 g. glucose, 2 to 3 ml. sulfuric acid, and 2 g. potassium acid sulfate heated to 150° for 2 hours for hydrazones and osazones. The glucose was increased to 0.5 g. and the time to 4 hours for heterocyclic azo compounds with two nitrogens in the ring. Others (5,35,51,159)

have used glucose or sucrose successfully for azo and aromatic nitro compounds.

Other reducing reagents which have been used with some success are salicylic acid plus thiosulfate, thiosalicylic acid, *o*-mercaptobenzoic acid (136), and even copper (80). The reaction of salicylic or thiosalicylic acid appears to be the liberation of sulfur dioxide rather than the formation of nitro derivatives which are more readily reduced than the sample material. Bradstreet (21) was able to recover only the original acid and no nitro acid derivative when using the salicylic acid–thiosulfate procedure. Thiosalicylic acid has usually been preferred to salicylic acid–thiosulfate by those who have compared the two reagents. These reagents have been used mostly in fertilizer analysis where inorganic nitrates are present, but can also be used for some organic nitrates and nitro compounds. Aliphatic materials respond better than aromatic, whereas the reverse is true for carbon reduction procedures.

The types of compounds which seem most difficult to analyze by the Kjeldahl method are aliphatic nitro, hydrazo, and chloronitrobenzenes. To date, no single method has been proposed by which these three types of compound can be analyzed successfully. The greatest need in Kjeldahl nitrogen analysis is a method for determining the nitrogen in all materials with N—O and N—N linkages which will produce good results under rigorous collaborative testing.

5. Closed Tube Digestion

Levi and Gimignani (87) in 1929 reported a closed-tube macro method, but the temperature was raised to only 330°C. and the digestion required as much time as the open-flask procedure. White and Long (176), however, demonstrated the practicality of using the closed-tube digestion technique for microanalysis. They used 5 to 10 mg. samples with 1.5 ml. sulfuric acid plus 40 mg. mercuric acid in sealed, heavy-walled, micro Carius tubes heated to 470°C. for 15 minutes in a specially constructed welded steel box placed in a muffle furnace. After cooling the tubes, the acid was transferred to a micro distillation apparatus and the ammonia determined in the usual manner. Compounds with N—O and N—N linkages gave low results.

Grunbaum *et al.* (45) showed that at temperatures over 500°C. there was some nitrogen loss, probably due to the decomposition of the ammonium acid sulfate or oxidation by sulfur trioxide or oxygen. The presence of a little water reduced the nitrogen loss and decreased the need for accurate temperature control. The temperature of 470°C. was considered dangerously high by Baker (6), who preferred 420 to 440°C. for 45 minutes.

He found that the nitrogen in N—O linkages could be determined quantitatively if *o*-mercaptobenzoic acid or glucose were added with the sample. N—N linkages did not yield to this treatment.

The method is subject to the same limitations as the open-flask digestion. This would be expected because it is primarily a way of obtaining the high temperatures required for the rapid digestion of refractory materials without adding a salt to raise the boiling point of the acid. It is advantageous if the ammonia is to be determined colorimetrically because the higher digestion temperature permits the elimination of the mercury catalyst which interferes in some procedures. However, the digestion time must be increased to compensate for the absence of a catalyst. Several good ultramicro methods (11,14,143) have been developed using the sealed-tube technique.

D. DISTILLATION, ABSORPTION, AND TITRATION OF AMMONIA

1. Distillation

The acidic digest is diluted and made alkaline by the careful addition of concentrated sodium hydroxide solution or sodium hydroxide pellets. The solution usually contains at least 30% sodium hydroxide plus sufficient sodium thiosulfate to precipitate all mercury present. Failure to precipitate all mercury will lead to low results because some ammonia will be retained as the nonvolatile $HgNH_2^+$ complex. Sodium or potassium sulfide may be added to precipitate the mercury, and these, like the thiosulfate, may be dissolved in the alkali solution or added separately.

In the macro method the alkali–thiosulfate solution must be added so that it runs down the side of the flask and under the diluted acid digest with a minimum of mixing. Unless care is exercised the whole solution may become basic and ammonia lost. Because the diluted acid digest and rinses are transferred to a steam distillation apparatus in the micro method, the danger of loss of ammonia is much reduced, but the ammonia absorbent may be drawn back into the apparatus if the mixing of the alkali–thiosulfate and acid solutions takes place too rapidly.

Enough alkali must be added to make the final solution sufficiently basic to permit the ammonia to be quantitatively and easily distilled. Too large an excess of alkali may lead to low nitrogen values when the solution contains precipitated mercuric sulfide. Too great an excess of alkali also increases the danger of obtaining high results in the macro method because of mechanical carryover of the alkaline spray formed above the

liquid in the distillation flask. Scrubber-type connecting bulbs reduce this source of error.

The limiting factor in the rate of ammonia distillation in microanalysis is the efficiency of the condenser. If the distillate coming from the condenser is hot, ammonia may be lost, particularly if boric acid is used as the ammonia absorbent. To insure quantitative distillation of the ammonia, it is advisable to collect a specified minimum volume of distillate rather than to distil for a fixed time because of possible variation in rate.

2. Ammonia Absorption

There has been a long-standing controversy as to whether boric acid or standard hydrochloric (or sulfuric) acid is the better absorbent for the ammonia. The advantages of using boric acid are: (*1*) The volume used does not need to be measured accurately; (*2*) only one standard solution (acid) need be prepared; and (*3*) if a little of the solution is lost before the ammonia starts to distill, the determination is not ruined. The advantages of a standard acid are: (*1*) No adjustment in the volume of the distillate before titration need be made; (*2*) loss of nitrogen is less likely if the distillation rate is too rapid for the condenser to cool the condensate completely; and (*3*) it seems to be a slightly more efficient absorbent (61).

Winkler (183) first proposed the use of boric acid in 1913 and since then it has been used by numerous workers (92,94,100,142,155,156,171), but more in micro- than in macroanalysis. A statistical study of the results of a collaborative test (61) showed that there was a significant difference between the results obtained with boric acid and those with standard hydrochloric or sulfuric acid, with the latter yielding slightly higher values. Although 4% boric acid solution is usually specified, it is more convenient to use a saturated solution which is maintained by keeping an excess of boric acid in the stock solution bottle. Because the pH of a boric acid solution increases as the solution is diluted, it is necessary, especially in microanalysis, to adjust the distillate to approximately the same volume before each titration.

The standard acids used are usually from 0.1 to 0.5*N* for macroanalysis and 0.01 or 0.02*N* for microanalysis. When these are used, the acid in excess of that required to neutralize the ammonia is titrated with a base of similar normality. The base is usually standardized against Bureau of Standard's potassium acid phthalate and the acid solutions against the standard base or against a primary standard, such as sodium carbonate prepared from sodium bicarbonate or against borax. The preparation and storage of these primary standards are described in *Official Methods of Analysis* of the AOAC. It is necessary to know the exact normality only

of the base because the calculation is based on the difference in the amount of base required to titrate a fixed volume of the acid with and without absorbed ammonia, or preferably the difference between a blank and a sample titration. It is advisable, however, as a double check on the whole titration and on the blank, to know the normality of the acid.

Potassium biiodate has been recommended (8,112) as an ammonia absorbent in microanalysis. Because this acid salt is used as a primary standard, the solution can be prepared by weighing the salt and diluting it to a known volume. The excess of acid after ammonia absorption can be determined by titration with base or with sodium thiosulfate after the addition of potassium iodide, and both can be standardized against the potassium biiodate solution. With the sharp end point obtainable with the iodometric titration and samples containing 0.1 to 1.4 mg. of nitrogen, Ballentine and Gregg (8) obtained a standard deviation of better than 0.01 for samples containing about 1% nitrogen.

3. Indicators

Although numerous acid–base indicators have been used in the titration, with methyl red being the most common, the mixed indicators methyl red–methylene blue (22) or methyl red–bromocresol green recommended by Ma and Zuazaga (92) are usually preferred. When mixed in the proper proportions, these cause the solution to pass through a gray or colorless stage at or near the equivalence point. The color change takes place over a much narrower pH range than with a single indicator, thus leading to a sharper, more reproducible end point. Methyl purple indicator solution, which contains methyl red and a blue dye and is available commercially has gained favor in recent years, particularly in macroanalysis. Lake *et al.* (82) preferred this indicator to methyl red, bromocresol green, or their mixture.

Sher (152), in a recent study of numerous possible indicators, points out that the equivalence point for ammonium chloride is pH 5.2, and that the gray stage for methyl red–methylene blue occurs at pH 5.3, the color change being from green to gray to purple. He also proposed a novel new indicator mixture consisting of bromocresol green, new coccine, and *p*-nitrophenol, which in boric acid changes progressively from green to blue to gray to yellow, with the green to blue change (pH 4.6) signalling the approach of the gray end point.

Several titration methods have been proposed which eliminate the distillation and acid–base titration. These are based on Kolthoff and Stenger's work (78) on the oxidation of ammonia to nitrogen with hypochlorite or hypobromite. In these procedures the acid digest is neutralized with

sodium hydroxide, and sodium bicarbonate, potassium bromide, and an excess of standard sodium hypochlorite are added. Belcher and Bhatty (10) then added an excess of standard arsenite solution and back-titrated with the standard hypochlorite, using tartrazine as indicator. Ashraf *et al.* (4) used the same procedure except that the indicator was Bordeaux B.

Ammonia in the distillate has also been determined (33) by oxidation with hypobromite and iodometric titration of the excess hypobromite after addition of potassium iodide and acetic acid. Hiller *et al.* (52) determined the nitrogen liberated by hypobromite oxidation gasometrically in Van Slyke manometric apparatus.

4. Calculations

It is essential that blank determinations be made to correct for any nitrogen in the reagents including the water used to dilute the acid digest and, in microanalysis, any ammonia present in the steam used to distil the ammonia. The water in the steam generator should be acidified, preferably with phosphoric acid, to eliminate this latter source of error. When using boric acid as absorbent, the blank also corrects for the acid necessary to bring the diluted boric acid solution to the end point.

Blanks should be run with some organic materials such as sucrose replacing the sample so that the traces of nitrate which are usually present will be reduced to a similar extent in both sample and blank analyses.

When boric acid is used, the volume of titrant equivalent to the nitrogen is $S - B$, where S and B (equation (1)) are the milliliters of standard acid required for the sample and blank, respectively. If a standard acid is used as absorbent, the volume of titrant used to calculate the nitrogen is $B - S$ and these represent ml. of standard base for blank and sample, respectively, equation (2).

For microanalysis using boric acid:

$$\frac{(S - B) \times N \times 14.007 \times 100}{\text{sample wt. (mg.)}} = \% \text{ nitrogen} \qquad (1)$$

For macroanalysis using standard acid:

$$\frac{(B - S) \times N \times 0.014007 \times 100}{\text{sample wt. (g.)}} = \% \text{ nitrogen} \qquad (2)$$

Since the normality is a constant, at least for each batch of standard solution, the terms $N \times 14.008 \times 100$ or $N \times 0.014007 \times 100$ are combined into a single factor to make the calculation simply:

$$\frac{\text{ml.} \times \text{factor}}{\text{sample wt.}} = \% N$$

In some laboratories the normality of the standard solution and the weight of sample are adjusted so that (factor)/(sample wt.) = 1, making the per cent nitrogen equal to the ml. of titrant. This may be feasible in control analysis where the per cent nitrogen is reasonably uniform, but the time required to obtain a specific weight of sample will likely be greater than that required for the simple calculation using a calculator.

E. EVALUATION OF KJELDAHL METHODS

1. Applications

The Kjeldahl method is applicable to the determination of nitrogen in most solid or liquid organic materials (see Section 2). Gaseous materials can be analyzed if the gas can be absorbed in a medium that does not contain nitrogen and is either digestible or volatile under the conditions of the analysis. Trace amounts of nitrogen in organic materials may be determined with reasonable accuracy. The digestion step is frequently more difficult because large amounts of some materials cause the digest to foam; with care in the digestion such materials as petroleum oils or honey may be analyzed satisfactorily. Lake (81), in a collaborative study on the determination of nitrogen in petroleum and shale oils, obtained coefficients of variation of 1.5% for oils with 1% nitrogen, and 4% for oils with 0.05 to 0.1% nitrogen with a macro method. With dilute aqueous solution or biological fluids, the water is merely distilled off during the initial part of the digestion. With samples containing large amounts of organic matter and trace of nitrogen, foaming can be reduced by carbonizing the sample, distilling out water or volatile solvent with half the required amount of sulfuric acid, then adding the catalyst, potassium sulfate, and the remainder of the acid prior to the usual digestion. The addition of a small amount of paraffin or a long-chain alcohol, such as cetyl or octadecyl, greatly reduces the foaming problem.

It must be remembered that when analyzing materials that contain cyanide or cyanate groups which can be hydrolyzed to hydrogen cyanide, the sulfuric acid must contain as little water as possible, otherwise low results will be obtained. The acid may be taken from a freshly opened bottle (173) or boiled just prior to use, but the simplest procedure is to add 1 part of fuming sulfuric acid to 2 parts of concentrated acid.

Comparative studies (81) have shown that under normal operating conditions macroanalysis is a little more precise, and therefore probably more accurate, than microanalysis. Both are, or can be, sufficiently accurate for most needs. Microanalysis has been gaining in popularity in recent years because of its saving in space, cost of equipment and reagents, and

amount of sample required. For control analysis of such materials as feeds or fertilizers where large numbers of analyses are required and plenty of sample is available, macroanalysis is still generally preferred. Homogeneity of sample is also a consideration that enters into the decision of whether the micro or macro method should be used. Samples sufficiently homogeneous for microanalysis can usually be obtained, but sometimes extra grinding and mixing are required and these steps require time. Material as difficult to mix as ground leather can be prepared for microanalysis by making three instead of one pass through a Wiley mill. The Wig-L-Bug vibratory mixer, used to mix potassium bromide and sample for infrared analysis by the pellet procedure, is a useful tool for homogenizing samples for microanalysis.

2. Limitations

As discussed earlier in this section, materials which contain N—O or N—N linkages must be pretreated to reduce the nitrogen or break the N—N bond before the sample is digested. Although many compounds of this type can be analyzed by some of the modified procedures already discussed, there is no universally applicable procedure for these materials. It is advisable to use the Dumas or ter Meulen method if the nature of the nitrogen linkages is unknown, or if poor results are obtained with test materials whose structure is similar to that of the sample.

The presence of large amounts of chlorides in samples containing nitrates, as in some fertilizers, may lead to low results. Recent efforts by the AOAC have been directed toward the development of a method for such materials. Fish and Collier (40) observed that low values were obtained by pyridinium iodides unless tin was added to the digest. They postulated that tin prevented the formation of oxyacids which are known to oxidize ammonia to nitrogen.

3. Precision and Accuracy

No procedure can really be considered to be a reliable quantitative method until it is shown that other analysts can obtain reliable results with it. This means that the method must be written so that the limits of all critical variables are defined and the chances for misinterpretation are minimized. The best critical evaluation is by collaborative testing as is done by the AOAC, ASTM, and other societies which establish official procedures. Collaborative tests must be well designed and samples should be preferably of known pure materials. These samples should present problems as adverse as will be encountered in normal operation and the

TABLE VI

Methods and Results of Collaborative Tests of the Micro Kjeldahl Method

Sample	Benzyl-isothiourea hydrochloride	Nicotinic acid	Nicotinic acid	Nicotinic acid	Tryptophan	Nicotinic acid	*N*-Octadecyl stearamide
Year	1949	1949	1951	1950	1950	1959	1959
ml. H_2SO_4	1.5	1.5	2	2	2	2 ± 0.1	2 ± 0.1
g. K_2SO_4	0.5	0.5	0.85	1.30	1.30	1.9 ± 0.1	1.9 ± 0.1
mg. HgO	40	40	40	40	40	40 ± 10	40 ± 10
Digestion (hr.)	1/2–1	1/2–1	4	4	1	1	1/2
Theoret. % N	13.82	11.38	11.38	11.38	13.72	11.38	2.59
No. collaborators	—[a]	—	14	14	16	23	23
Interlab. mean[c]	13.80	—[b]	10.27	11.34	13.66	11.34	2.62
Interlab. S. D.	—	—[b]	—[b]	0.109	—	0.100	0.081
Intralab. S. D.[d]	—	—[b]	—[b]	0.114	—	0.03	0.04

[a] A total of 23 determinations were made in several laboratories and the S. D. for all values was 0.122.

[b] Data not worth analyzing.

[c] Interlaboratory mean is average of individual laboratory means.

[d] Intralaboratory standard deviation is average of S. D. for participating laboratories.

results should be analyzed statistically so that at least the interlaboratory precision is known. If this precision is poor, either the method is not good or the author of the method has failed to describe it adequately. The present micro Kjeldahl method of the AOAC was established by several collaborative studies. The results of some of these tests are shown in Table VI.

From the results obtained for benzylisothiourea hydrochloride in 1949, the method studied might be considered satisfactory, but this was not true for the refractory nicotinic acid, which provides a more critical test. In the latter case the results showed that a really satisfactory method was not available until the temperature of the digestion was increased by a higher salt/acid ratio.

Similar studies on the present AOAC macro method (30) have shown that the average intralaboratory standard deviation for analyses performed by the same laboratory one week apart was 0.06%, using nicotinic acid as the test material. Net interlaboratory standard deviation (corrected for intralaboratory variations) was 0.12, which is lower than it would be if the usual calculation had been used. The corresponding values for a urea–formaldehyde material (approximately 38% N) were 0.17 and 0.21. The interlaboratory standard deviation for nicotinic acid by the macro method was higher than that calculated for the micro method. However, the data for the macro method were obtained from analyses performed on two separate days one week apart, whereas the microanalytical data were probably all obtained on the same day. The mean value for both macro- and microanalyses was 11.34, or 0.04% below the theoretical value.

4. Space Requirements

One of the advantages of the micro method is the saving in space. The circular 12-unit digestion rack shown in Fig. 10 requires only about 2 feet of bench space, the distillation unit plus titration equipment requires another 3 to 4 feet, and all equipment is easily moved. Storage space for digestion and titration flasks is also minimal. Two electrical outlets, a normal laboratory water aspirator or vacuum line, and a cold water tap are also needed.

The macro equipment, on the other hand, requires wall space of about 7 feet for a 12-unit combined digestion–distillation rack, or twice this space for separate units. In addition, at least 3 to 4 feet of bench space is required for titration, and the usual storage of digestion flasks in racks or carts requires an additional 2 to 4 feet of bench or wall space. Also required are a large diameter water line and drain or a fume duct to the out-

side of the building to remove the fumes, and a separate water line for the condensers. A high amperage electrical line (or a gas line) for the digestion and distillation steps completes the major services for the macro method.

III. RECOMMENDED LABORATORY PROCEDURES

A. MICRO METHOD*

1. Reagents

(*a*) *Sulfuric acid.* Sp. gr. 1.84, N-free.

(*b*) *Mercuric oxide.* Reagent grade, N-free.

(*c*) *Potassium sulfate.* Reagent grade, N-free.

(*d*) *Sodium hydroxide–sodium thiosulfate solution.* Dissolve 60 g. NaOH and 5 g. $Na_2S_2O_3 \cdot 5H_2O$ in H_2O and dilute to 100 ml. or add 25 ml. 25% $Na_2S_2O_3 \cdot 5H_2O$ to 100 ml. 50% NaOH solution.

(*e*) *Boric acid solution.* Saturated solution.

(*f*) *Indicator solution.* (*1*) Methyl red–methylene blue: Mix 2 parts 0.2% alcoholic methyl red solution with 1 part 0.2% alcoholic methylene blue solution; or (*2*) methyl red–bromocresol green solution: Mix 1 part 0.2% alcoholic methyl red solution with 5 parts 0.2% alcoholic bromocresol green solution.

(*g*) *Hydrochloric acid.* 0.01 or 0.02*N*. Standardized against a primary standard.

2. Apparatus

(*a*) *Digestion rack.* Use rack with electric heaters adjusted to supply sufficient heat to 30-ml. flask to cause 15 ml. H_2O at 25° to come to rolling boil in not less than 2 or more than 3 minutes.

(*b*) *Distillation apparatus.* Use one-piece, Fig. 11, or Parnas-Wagner, Fig. 12, distillation apparatus.

(*c*) *Digestion flasks.* Use 30-ml. regular Kjeldahl or Solty's type flasks. For small samples 10-ml. Kjeldahl flasks may be used.

3. Determination

Weigh sample requiring 3 to 10 ml. 0.01 or 0.02*N* HCl and transfer to digestion flask. Sample should not contain more than 100 mg. dry organic matter. Use charging tube for dry solids, porcelain boat for sticky solids or nonvolatile liquids, and glass capillary or capsule for volatile liquids. Add 1.9 ± 0.1 g. K_2SO_4, 40 ± 10 mg. HgO, and 2.0 ± 0.1 ml. H_2SO_4.

* Not applicable to material containing N—N or N—O linkages.

If sample weight is more than 15 mg., add additional 0.1 ml. H_2SO_4 for each 10 mg. dry organic matter over 15 mg. Use mixture of 1 part fuming and 2 parts concentrated acid if sample contains nitriles. Ten-milliliter flasks and half quantities of reagents may be used for samples smaller than 7 mg. Add boiling chips which pass No. 10 sieve. If boiling time for digestion rack heaters is 2 to 2.5 minutes, digest 1 hour after all H_2O is distilled and acid comes to true boil; if boiling time is 2.5 to 3 minutes, digest 1.5 hours. (Digest 30 minutes if sample is known to contain no refractory ring N.)

Cool, add minimum quantity of H_2O to dissolve solids, cool, and place thin film of Vaseline on rim of flask. Transfer digest and boiling chips to distillation apparatus and rinse flask 5 or 6 times with 1 to 2-ml. portions of H_2O. Place 125-ml. Phillips beaker or Erlenmeyer flask containing 5 ml. saturated H_3BO_3 solution and 2 or 3 drops indicator under condenser with tip extending below surface of solution. Add 8 ml. $NaOH$–$Na_2S_2O_3$ solution to still, collect at least 15 ml. distillate, and dilute to 50 ml. (Use 2.5 ml. H_3BO_3 and 1 or 2 drops indicator, and dilute to 25 ml. if 0.01*N* HCl is to be used.) Titrate to gray end point or first appearance of color after gray. Make blank determination with 5 to 10 mg. glucose or sucrose and calculate % N.

4. Notes

The hydrochloric acid solution may be standardized against tris(hydroxymethyl) aminomethane, sodium carbonate prepared from sodium bicarbonate, or borax prepared and preserved as described in *Official Methods of Analysis* of the AOAC. They also may be prepared by diluting 0.1*N* acid standardized against one of the primary standards.

It is sometimes necessary to adjust the ratio of the indicators to obtain the optimum mixture.

Solty's flasks are recommended for materials which bump badly.

The final salt/acid ratio (g./ml.) should not exceed 1.1:1.

Nicotinic acid should be used as a standard test sample.

Standard acid solution may be used instead of boric acid to absorb the ammonia and the excess acid titrated with standard base.

If sample foams badly, add 2 to 3 mg. cetyl or similar alcohol and digest at reduced heat until foaming ceases.

Titration vessels may be marked at 25 and 50 ml. levels and distillate diluted to these marks before titration.

B. MACRO METHOD*

* Not applicable to material containing N—N or N—O linkages.

1. Reagents

(*a*) *Sulfuric acid.* Sp. gr. 1.84, N-free.

(*b*) *Mercuric oxide.* Reagent grade, N-free.

(*c*) *Potassium sulfate.* Reagent grade, N-free.

(*d*) *Sodium hydroxide–sodium thiosulfate solution.* Dissolve 600 g. NaOH and 50 g. $Na_2S_2O_3 \cdot 5H_2O$ in H_2O and dilute to 1000 ml., or add 250 ml. 25% $Na_2S_2O_3 \cdot 5H_2O$ to 1000 ml. 50% NaOH solution.

(*e*) *Hydrochloric or sulfuric acid.* 0.1*N* (or 0.2*N*) standardized against a primary standard.

(*f*) *Sodium hydroxide.* 0.1*N*, made by diluting 50% sodium hydroxide with CO_2-free water and standardized against potassium acid phthalate standard.

(*g*) *Indicator solution.* Mix 2 parts 0.2% alcoholic methyl red solution with 1 part 0.2% alcoholic methylene blue solution, or use commercial methyl purple indicator solution.

(*h*) *Zinc granules.* Reagent grade.

2. Apparatus

(*a*) *Digestion rack.* Use rack, preferably with electric heaters, adjusted to bring 250 ml. of water from 25°C. to a rolling boil in 4 to 6 minutes. Preheat heaters and add boiling chips to prevent superheating.

(*b*) *Distillation rack.* Use rack with electric or gas heaters and with efficient scrubber type traps to prevent mechanical carryover of alkaline spray.

(*c*) *Kjeldahl flasks.* Use 500, 650, or 800-ml. standard Kjeldahl flasks of borosilicate glass. Flask size depends on sample size and amount of foaming expected.

3. Determination

Weigh sample equivalent to 10 to 40 ml. of 0.1*N* (or 0.2*N*) acid and transfer to digestion flask. Keep sample weight between 0.5 and 1.5 g. if possible. Add 18 ± 0.5 g. K_2SO_4, 1.4 ± 0.1 g. HgO, and 25 ± 0.5 ml. H_2SO_4. If sample exceeds 1.5 g., add additional 1 ml. H_2SO_4 for each 0.1 g. dry organic matter over 1.5 g. Add boiling chips and digest 1 hour after water is distilled and acid comes to true boil, or digest 30 minutes if sample is known to contain no refractory ring nitrogen. (Digestion time after clearing should approximately equal boiling time required to clear digest.)

Cool, add approximately 200 ml. water, mix to dissolve all K_2SO_4, and cool to room temperature. Carefully add 70 ml. of the sodium hydroxide–sodium thiosulfate solution with the flask held at an angle so that

the alkali solution forms a layer at the bottom of the flask. Add approximately 0.7 g. zinc granules, and connect flask to trap and condenser immediately. Place 500-ml. wide-mouth Erlenmeyer flask or Phillips beaker containing 50 ml. standard acid and 3 or 4 drops indicator solution under condenser with tip immersed in acid solution. Rotate Kjeldahl flask to mix contents and distill until approximately 150 ml. condensate are collected. Titrate excess standard acid with standard alkali solution. Make blank determination with glucose or sucrose added and calculate % N.

4. Notes

The final salt acid ratio should not exceed 1.1:1. Consequently, with samples such as fatty materials that are high in carbon and hydrogen content, more acid than specified may be required, or the samples held to less than 1 g.

When samples are of similar composition the amount of salt or acid added should be adjusted to give a final salt acid ratio of approximately 1:1.

If heaters cannot be adjusted to give a boiling time as low as 4 to 6 minutes, the digestion time will have to be increased.

Nicotinic acid should be used as a standard sample to test procedure and set digestion time.

Thirty grams of sodium hydroxide pellets and approximately 4 g. solid sodium thiosulfate may be used in place of the sodium hydroxide–sodium thiosulfate solution.

For samples that foam badly, digest at reduced heat until foaming is reduced. A small amount of paraffin or long-chain alcohol helps to reduce the foaming.

Standard acid may be replaced by 50 ml. of 4% or saturated boric acid and the ammonia titrated with 0.1*N* (or 0.2*N*) standard acid. If boric acid is used, the volume should be adjusted to about 250 ml. before titration of the ammonia.

Part 3

Other Methods. By Clyde L. Ogg

I. AUTOMATED KJELDAHL ANALYSIS

No chapter on nitrogen methods would be complete without a discussion of the present developments leading to automation of the Kjeldahl procedure. Technicon Instruments Corporation has completely automated

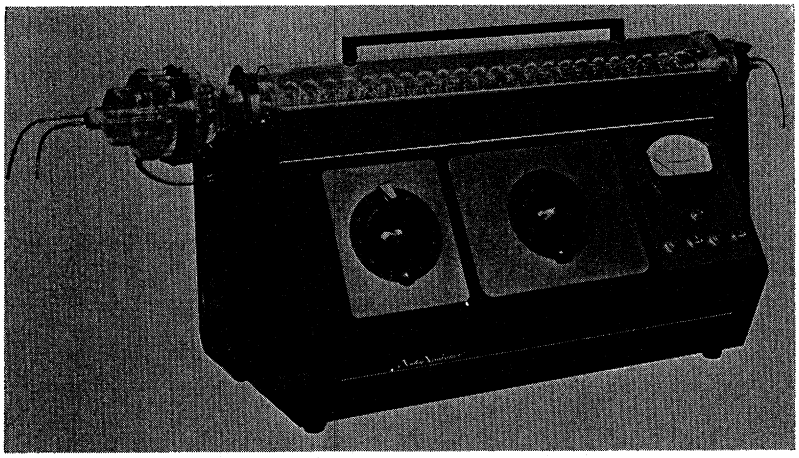

Fig. 13. Digestion unit for automated Kjeldahl analysis.

the Kjeldahl method from the removal of an aliquot of a sample to the presentation of an absorption curve for the color developed by the ammonia. The sample aliquot is mixed with a metered amount of sulfuric acid containing dissolved catalyst and digested in a revolving glass spiral container heated by a furnace. The digest is diluted, sampled, neutralized, color developed by the phenoxide–hypochlorite colorimetric method, and the absorbance at 630 mμ recorded. The maximum analysis rate is 20 determinations per hour, but this rate sacrifices some accuracy for speed. Maximum accuracy is attained at about 7 analyses per hour. About 12 per hour seems to be optimum.

The development and measurement of color in solutions by the Technicon's AutoAnalyzer has been in use for several years for various determinations. Adaptation of these units to the Kjeldahl method required the development of a digestion apparatus which would operate on a flow principle. The digestor developed is shown in Fig. 13 and is essentially a long glass tube with spiral evolutions mounted over electric heating elements. The temperature of the air just below the tube may be varied over a wide range but is usually maintained between 400 and 450°C. The sample and digestant are fed in at one end continuously during the sampling period and move down the tube as it is rotated. The rate of rotation controls the digestion time which is 3 to 4 minutes but can be as low as 1.5 minutes. At the exit end the sample is first diluted, then removed by suction into a mixing cup, where an aliquot of the digest is taken for colorimetric analysis. The digestant recommended is 90% sulfuric acid containing selenium oxychloride and perchloric acid; however, a mercuric salt can be used. Inter-

ference of the mercury salt with phenoxide–hypochlorite color reaction can be eliminated by adding EDTA to the sodium hydroxide solution used to neutralize the acid (39).

Two questions immediately arise: First, how can the sample be digested so quickly; and second, why does not the selenium and perchloric acid cause nitrogen loss at these high digestion temperatures? The answers appear to be: First, with the sample and acid digestant mostly distributed as a thin film on the hot glass spiral, the rate of digestion is much more rapid than when they are heated as a body of fluid in a flask; and second, the selenium and perchloric acid do not cause nitrogen loss because of the short digestion time. Even refractory materials like nicotinic acid and nicotinamide yield quantitative results under the conditions described above. The original design required that a sample be either a liquid or a solution and this excluded the analysis of feeds, most fertilizers, and many other materials. A novel system has now been developed by Ferrari (39) for analyzing solid materials. The sample is weighed in a plastic cup which is placed on a revolving disk, then automatically emptied into a funnel and rinsed into a blender with a measured amount of water or other liquid. After being homogenized in the blender, an aliquot is removed by the apparatus and fed into the digester with the sulfuric acid and catalyst.

The automated process is still subject to the usual limitations of the Kjeldahl method. Materials with N—O and N—N bonds must be treated to reduce the nitrogen before digestion. Work is now being conducted to develop a reduction procedure which will be applicable to the automated method.

It is difficult at this time to assess fairly the accuracy and precision of the method because it is so new and because improvements are still being made in the process, particularly as to the sampling of solid samples. Standard deviations of better than 0.1% and recoveries of 100 ± 1 or 2% are reported to be readily attainable at the present time. The accuracy will most likely improve as more experience is gained and it seems likely that the automated equipment will replace the conventional Kjeldahl apparatus in many laboratories where large numbers of nitrogen analyses are required.

II. COLORIMETRIC METHODS

That there is need for a more rapid or multiple-unit micro Kjeldahl distillation apparatus is shown by the relatively large number of micro colorimetric methods reported in recent years. In addition to the saving in time when analyzing a large number of samples, the colorimetric methods have the advantage of permitting the determination of small amounts of ammonia more accurately than the distillation and titration procedure.

The reaction of phenol or sodium phenoxide, hypochlorite, and ammonium ion to form indophenol blue was first reported by Berthelot (15) and has been used to determine the ammonia in the digest by several workers. Van Slyke and Hiller (174) preferred sodium phenoxide–hypochlorite to Nessler's reagent because the colored product was truly soluble while the sensitivity was equivalent. The color reaction was modified by Riley (136) and Bohnstedt (16) by the addition of manganous sulfate. The color was developed by heating at 70°C. for 45 minutes and the solution then cooled, allowed to stand 30 minutes, and read at 600 mμ. Riley applied the color method to the determination of ammonia in natural and sea waters and found that Beer's law was obeyed up to 2 mg. of nitrogen per liter. Noble (113) and Milner *et al.* (105) used the phenoxide–hypochlorite method to determine traces of ammonia in the distillate in the analysis of petroleum stocks. Noble claimed a detectable limit of 1 p.p.m. with 5 g. samples, whereas Milner obtained a standard deviation of 0.14 p.p.m. in the 0 to 3 p.p.m. range by extracting 70-g. samples with dilute sulfuric acid. The water and sulfuric acid were redistilled and the potassium sulfate used in the digestion was heated to more than 550°C. to remove traces of ammonia. Both used phenol and commercial Clorox and heated 6 to 8 minutes in boiling water to develop the color which was then measured spectrophotometrically at 610 mμ. Lubochinsky and Zalta (89) neutralized the acid digest with sodium hydroxide to the methyl red end point, added sodium phenoxide, phosphate buffer to pH 12, sodium nitroprusside and hypochlorite, then allowed the solution to stand for 30 minutes in the dark before reading at 610 mμ.

Nessler's reagent has been used by numerous authors (83,88,95,104) to determine the ammonia in the Kjeldahl digest but application has been limited to easily digested materials because mercury cannot be used as catalyst. Most procedures have specified sulfuric acid and hydrogen peroxide for digestion. Lang (83), using selenium oxychloride, reported fairly good results for most materials studied, but tryptophan values were 10% low. This points up the weakness in the methods using Nessler's reagent, which is that low results may be obtained because the digestion temperatures are too low and mercury catalyst cannot be used. Of the several ways of preparing Nessler's reagent, that described by Koch and McMeekin (77) in 1924 is still used extensively. The preparation described in *Reagent Chemicals, American Chemical Society Specifications, 1960*, p. 25 (1) is easier to carry out (99) and the reagent is equally as good. Nessler's reagent has the following two advantages over phenoxide–hypochlorite: First, the alkalinity of the reagent is frequently sufficient to neutralize the acid digest so that only one reagent need be added; and

second, no heating is required to develop the color. Jacobs (59,60) has proposed ninhydrin as a color reagent for the ammonia in the Kjeldahl digest. After digestion, the acid solution was neutralized, diluted with buffer solution, ninhydrin reagent added, the mixture heated for 30 minutes in a boiling water bath, cooled, diluted, and read at 570 mμ. Potassium sulfate and mercury catalyst were used in the digestion, permitting the analysis of refractory materials. This colorimetric procedure was coupled with the sealed-tube digestion technique to give an accurate ultramicro method.

With the use of EDTA to eliminate the interference of mercury in the phenoxide–hypochlorite method, as recommended by Ferrari (39), either this method or Jacobs' ninhydrin procedure may be used to determine the ammonia in digests containing mercury catalyst.

III. TER MEULEN METHOD

The ter Meulen method (101,102) offers another way of converting nitrogen in organic materials to ammonia which can be either titrated or determined colorimetrically. In this procedure the material is pyrolyzed in an atmosphere of hydrogen and the vaporized products passed over nickel–magnesium catalyst at 320°C. Pyrolysis and reduction of the nitrogen to ammonia is carried out in a combustion apparatus similar to that used for carbon and hydrogen analysis. The ammonia is absorbed in boric acid if it is to be titrated with standard acid, or in acidified water for colorimetric determination.

Before the work of Holowchak *et al.* (55), the life of the catalyst was too short to make the method practical when other methods could be used. The catalyst prepared by these workers, however, has a life of over 100 analyses and makes the method worthy of serious consideration.

The principal application has been in petroleum analysis where the need is for the accurate determination of trace amounts of nitrogen. In addition to its use in this field by Holowchak, King, and Faulconer (69), using Nessler's reagent, claimed a sensitivity of 1 p.p.m. and an accuracy and precision of about 1 p.p.m. for petroleum samples containing 20 p.p.m. or less of nitrogen. Schluter (144), also using Holowchak's catalyst, absorbed the nitrogenous materials from petroleum stocks on silica gel, inserted the silica gel in the combustion tube, carried out the pyrolysis and reduction, and determined the ammonia either titrimetrically or colorimetrically, depending upon the amount of nitrogen present.

One of the advantages of the method is that all nitrogen, including that in N—O and N—N linkages, is quantitatively converted to ammonia. Another desirable feature is that the method is especially good for de-

termining traces of nitrogen. The accuracy and precision of the ter Meulen method are comparable to those for the Kjeldahl method for most materials, and frequently better than the Kjeldahl method for materials with traces of nitrogen and with N—O and N—N linkages. One disadvantage of the method is that it is not as easily adaptable to a large number of simultaneous analyses as the Kjeldahl method; however, with multiple, mechanized, pyrolysis–reduction units a considerable number of analyses could be made per day. Most of the applications in this country have been in petroleum analysis where relatively large samples are used for the determination of traces of nitrogen. It is a much more popular method in Europe, where it is frequently used for more conventional microanalysis.

REFERENCES

1. *Reagent Chemicals; American Chemical Society Specifications,* **1960.** American Chemical Society, Applied Publications, Washington, D. C., 1961.
2. American Society for Testing Materials, ASTM, *Designation, E 147-59T* (1959).
3. Asami, T., *Anal. Chem.*, **31**, 630 (1959).
4. Ashraf, M., M. K. Bhatty, and R. A. Shah, *Pakistan J. Sci. Ind. Res.*, **3**, 1 (1960).
5. Ashraf, M., M. K. Bhatty, and R. A. Shah, *Anal. Chim. Acta,* **25**, 448 (1961).
6. Baker, P. R. W., *Analyst,* **80**, 481 (1955).
7. Baker, P. R. W., *Talanta,* **8**, 57 (1961).
8. Ballentine, R., and J. R. Gregg, *Anal. Chem.*, **19**, 281 (1947).
9. Beet, A. E., *Sheffield Univ. Fuel Soc. J.*, **6**, 12 (1955); through *Anal. Abstr.*, **3**, 1039 (1956).
10. Belcher, R., and M. K. Bhatty, *Mikrochim. Acta,* **1956**, 1183.
11. Belcher, R., R. L. Bhasin, and T. S. West, *J. Chem. Soc.*, **1959**, 2585.
12. Belcher, R., and A. L. Godbert, *J. Soc. Chem. Ind.*, **60**, 196 (1941).
13. Belcher, R., and R. Goulden, *Mikrochem. Mikrochim. Acta,* **36/37**, 679 (1951).
14. Belcher, R., T. S. West, and M. Williams, *J. Chem. Soc.*, **1957**, 4323.
15. Berthelot, M., *Repertoire de chimie appliquée,* **1**, 284 (1859).
16. Bohnstedt, U., *Z. Anal. Chem.*, **163**, 415 (1958).
17. Bradstreet, R. B., *Chem. Rev.*, **27**, 331 (1940).
18. Bradstreet, R. B., *Anal. Chem.*, **26**, 185 (1954).
19. Bradstreet, R. B., *Anal. Chem.*, **26**, 235 (1954).
20. Bradstreet, R. B., *Anal. Chem.*, **29**, 944 (1957).
21. Bradstreet, R. B., *Anal. Chem.*, **32**, 114 (1960).
22. Brecher, C., *Wien. Klin. Wochschr.*, **49**, 1228 (1936).
23. Browne, E. J., and J. B. Polya, *Anal. Chem.*, **34**, 298 (1962).
24. Cepciansky, I., and L. Chromcova, *Sb. Ved. Praci, Vysoka Skola Chem.-Technol. Pardubice,* **1959**, 281.
25. Clark, E. P., *J. Assoc. Offic. Agr. Chemists,* **16**, 575 (1933).
26. Clark, E. P., *Semimicro Quantitative Organic Analysis,* Academic Press, New York, 1943.

26a. Clerc, J. T., and W. Simon, *Microchem. J.*, **7**, 422 (1963).

27. Coleman Instrument Co., Maywood, Illinois, *Operating Directions for Model* **29** *Coleman Nitrogen Analyzer,* D292-B (Dec. 1960).

28. Coulombeau, J., and E. Jaudon, *Chim. Anal.*, **42** (2), 61 (1960); through *Anal. Abstr.*, **7**, 4282 (1960).
29. Dalrymple, R. S., and G. B. King, *Ind. Eng. Chem., Anal. Ed.*, **17**, 403 (1945).
30. Davis, H. A., and S. R. Miles, *J. Assoc. Offic. Agr. Chemists*, **39**, 550 (1956).
31. Davis, C. F., and M. Wise, *Cereal Chem.*, **10**, 488 (1933).
32. Dickinson, W. E., *Anal. Chem.*, **30**, 992 (1958).
33. Dixon, J. P., *Anal. Chim. Acta*, **13**, 12 (1955).
34. Dorfman, L., R. Oeckinghaus, F. Anderson, and G. I. Robertson, *Anal. Chem.*, **34**, 678 (1962).
35. Elek, A., and H. Sobotka, *J. Am. Chem. Soc.*, **48**, 501 (1926).
36. Esafov, V. I., *Zavodsk. Lab.*, **21**, 1160 (1955).
37. Evans, W. A., F. B. Johnston, and G. M. Ward, *Lab. Practice*, **8**, 174 (1959).
38. Fawcett, J. K., *J. Med. Lab. Technol.*, **12**, 1 (1954).
39. Ferrari, A., personal communication.
40. Fish, V. B., and P. R. Collier, *Anal. Chem.*, **30**, 151 (1958).
41. Francis, H., personal communication.
42. Friedrich, A., E. Kühaas, and R. Schnurcn, *Z. Physiol. Chem.*, **216**, 68 (1933).
43. Gawargious, Y. A., and A. M. G. Macdonald, in N. D. Cheronis, Ed., *Proc. International Symposium on Microchemical Techniques*, Interscience, New York, 1962.
44. Gerritz, H. W., and J. L. St. John, *Ind. Eng. Chem., Anal. Ed.*, **7**, 380 (1935).
45. Grunbaum, B. W., P. L. Kirk, L. G. Green, and C. W. Koch, *Anal. Chem.*, **27**, 384 (1955).
46. Gunning, J. W., *Z. Anal. Chem.*, **28**, 188 (1889).
47. Gustin, G. M., *Microchem. J.*, **1**, 75 (1957).
48. Gustin, G. M., *Microchem. J.*, **4**, 43 (1960).
49. Gutbier, G., and M. Boëtius, *Mikrochim. Acta*, **1960**, 636.
50. Harrel, C. G., and J. H. Lanning, *Cereal Chem.*, **6**, 72 (1929).
51. Harte, R. A., *Ind. Eng. Chem., Anal. Ed.*, **7**, 432 (1935).
52. Hiller, A., J. Plazin, and D. D. Van Slyke, *J. Biol. Chem.*, **176**, 1401 (1948).
53. Hilton, C., personal communication.
54. Hofstader, R. A., personal communication.
55. Holowchak, J., G. E. C. Wear, and E. L. Baldeschwieler, *Anal. Chem.*, **24**, 1754 (1952).
56. Hozumi, K., and S. Kinoshita, *J. Pharm. Soc. Japan*, **76**, 1157 (1956); through *Anal. Abstr.*, **4**, 2993 (1957).
57. Hozumi, K., K. Imaeda, and S. Kinoshita, *J. Pharm. Soc. Japan*, **76**, 1161 (1956); through *Anal. Abstr.*, **4**, 2993 (1957).
58. Hozumi, K., and W. J. Kirsten, *Anal. Chem.*, **34**, 434 (1962).
59. Jacobs, S., *Nature*, **183**, 262 (1959).
60. Jacobs, S., *Analyst*, **85**, 257 (1960).
61. Joint Committee of Assoc. Offic. Agr. Chemists and Am. Oil Chemists' Soc., *J. Am. Oil Chemists' Soc.*, **32**, 35 (1955).
62. Kainz, G., *Oesterr. Chemiker-Ztg.*, **57**, 242 (1956).
63. Kainz, G., and H. Horwatitsch, *Z. Anal Chem.*, **175**, 272 (1960); through *Anal. Abstr.*, **8**, 607 (1961).
64. Kainz, G., and H. Horwatitsch, *Z. Anal Chem.*, **176**, 17 (1960); through *Anal. Abstr.*, **8**, 606 (1961).
65. Kainz, G., and H. Horwatitsch, *Mikrochim. Acta*, **1962**, 7.
66. Kainz, G., and F. Kasler, *Z. Anal. Chem.*, **168**, 425 (1959); through *Anal. Abstr.*, **7**, 1773 (1960).

67. Kakabadse, G., and B. Manohin, *Analyst*, **86**, 512 (1961).
68. Kaye, I. A., and N. Weiner, *Ind. Eng. Chem., Anal. Ed.*, **17**, 397 (1945).
69. King, R. W., and W. B. M. Faulconer, *Anal. Chem.*, **28**, 255 (1956).
70. Kirk, P. L., *Ind. Eng. Chem., Anal. Ed.*, **8**, 223 (1936).
71. Kirk, P. L., *Anal. Chem.*, **22**, 354 (1950).
72. Kirk, P. L., *Quantitative Ultramicroanalysis*, Wiley, New York, 1950.
73. Kirsten, W., "The Dumas Determination of Nitrogen," in C. L. Wilson, and D. W. Wilson, Eds., *Comprehensive Analytical Chemistry*, Elsevier, Amsterdam, 1960, Vol. IB, pp. 467–494.
74. Kirsten, W. J., and B. W. Grunbaum, *Anal. Chem.*, **27**, 1806 (1955).
75. Kirsten, W., and B. Wallberg-Olausson, *Anal. Chem.*, **23**, 927 (1951).
76. Kjeldahl, J., *Z. Anal. Chem.*, **22**, 366 (1883).
77. Koch, F. C., and T. L. McMeekin, *J. Am. Chem. Soc.*, **46**, 2066 (1924).
78. Kolthoff, I. M., and V. A. Stenges, *Ind. Eng. Chem., Anal. Ed.*, **7**, 79 (1935).
79. Kuck, J. A., and P. L. Altieri, *Mikrochim. Acta*, **1954**, 17.
80. Kuznetsov, V. I., *Zavodsk. Lab.*, **9**, 1039 (1940).
81. Lake, G. R., *Anal. Chem.*, **24**, 1806 (1952).
82. Lake, G. R., P. McCutchan, R. Van Meter, and J. C. Neel, *Anal. Chem.*, **23**, 1634 (1951).
83. Lang, C. A., *Anal. Chem.*, **30**, 1692 (1958).
84. Lange, N. A., *Handbook of Chemistry*, 10th ed., McGraw-Hill, New York, 1961, pp. 1681–1685.
85. Lauro, M. F., *Ind. Eng. Chem., Anal. Ed.*, **3**, 401 (1931).
86. Lepper, W., *Landw. Vers.-Sta.*, **111**, 155 (1930).
87. Levi, T. G., and L. Gimignani, *Gazz. Chim. Ital.*, **59**, 757 (1929).
88. Levy, M., *Compt. Rend. Trav. Lab. Carlsberg*, **21**, 101 (1936).
89. Lubochinsky, B., and J. P. Zalta, *Bull. Soc. Chim. Biol.*, **36**, 1363 (1954).
90. Ma, T. S., and M. Gutterson, *Anal. Chem.*, **34**, 111R (1962).
91. Ma, T. S., R. E. Lang, and J. D. McKinley, Jr., *Mikrochim. Acta*, **1957**, 368.
92. Ma, T. S., and G. Zuazaga, *Ind. Eng. Chem., Anal. Ed.*, **14**, 280 (1942).
93. Macdonald, A. M. G., *Ind. Chem.*, **31**, 144 (1955).
94. Machemer, P. E., and W. M. McNabb, *Anal. Chim. Acta*, **3**, 428 (1949).
95. Mácola, B. A., and C. Fazio, *Analyst*, **66**, 448 (1941).
96. Malter, L., personal communication.
96a. Maresh, C., O. E. Sundberg, R. A. Hofstader, and G. E. Gerhardt, in N. D. Cheronis, ed., *Microchemical Techniques*, Vol. 2, Interscience, New York, 1962, p. 387.
97. Marino, S. P., *Chemist Analyst*, **39**, 42 (1950).
98. McKenzie, N. A., and H. S. Wallace, *Australian J. Chem.*, **7**, 55 (1954).
99. McMeekin, T. L., personal communication.
100. Meeker, E. W., and E. C. Wagner, *Ind. Eng. Chem., Anal. Ed.*, **5**, 396 (1933).
101. Meulen, H. ter, *Rec. Trav. Chim.*, **43**, 643 (1924).
102. Meulen, H. ter, and J. Heslinga, *Rev. Prod. Chim.*, **30**, 407 (1927).
103. Milbauer, J., *Z. Anal. Chem.*, **111**, 397 (1938).
104. Miller, G. L., and E. E. Miller, *Anal. Chem.*, **20**, 481 (1948).
105. Milner, O. I., R. J. Zahner, L. S. Hepner, and W. H. Cowell, *Anal. Chem.*, **30**, 1528 (1958).
106. Milner, R. T., and M. S. Sherman, *Ind. Eng. Chem., Anal. Ed.*, **8**, 331 (1936).
107. Milton, R. F., and W. A. Waters, *Methods of Quantitative Micro-analysis*, 2nd Ed., Arnold, London, 1955.

108. Mizukami, S., and K. Miyahara, *Mikrochim. Acta*, **1962**, 705.
109. Mott, R. A., and H. C. Wilkinson, *Fuel*, **37**, 151 (1958).
110. National Research Council *International Critical Tables*, Vol. 3, McGraw-Hill, New York, 1928, p. 373.
111. Niederl, J. B., and V. Niederl, *Micro Methods of Quantitative Organic Analysis*, 2nd Ed., Wiley, New York, 1942.
112. Niederl, J. B., V. Niederl, and M. Eitingon, *Mikrochemie*, **25**, 143 (1938).
113. Noble, E. D., *Anal. Chem.*, **27**, 1413 (1955).
114. *Official and Tentative Methods of Analysis*, 5th Ed., Association of Official Agricultural Chemists, Washington, D. C., 1940.
115. *Official Methods of Analysis*, 9th Ed., Association of Official Agricultural Chemists, Washington, D. C., 1960.
116. Ogg, C. L., *J. Assoc. Offic. Agr. Chemists*, **43**, 689 (1960).
117. Ogg, C. L., R. W. Brand, and C. O. Willits, *J. Assoc. Offic. Agr. Chemists*, **31**, 663 (1948).
118. Ogg, C. L., and C. O. Willits, *J. Assoc. Offic. Agr. Chemists*, **33**, 100 (1950).
119. Ogg, C. L., and C. O. Willits, *J. Assoc. Offic. Agr. Chemists*, **35**, 288 (1952).
120. Osborn, R. A., and A. Krasnitz, *J. Assoc. Offic. Agr. Chemists*, **16**, 110 (1933).
121. Osborn, R. A., and A. Krasnitz, *J. Assoc. Offic. Agr. Chemists*, **17**, 339 (1934).
122. Osborn, R. A., and J. B. Wilkie, *J. Assoc. Offic. Agr. Chemists*, **18**, 604 (1935).
123. Parnas, J. K., and R. Wagner, *Biochem. Zeit.*, **125**, 253 (1921).
124. Parri, W., *Giorn. Farm. Chim.*, **71**, 253 (1922).
125. Patel, S. M., and A. Sreenivasan, *Anal. Chem.*, **20**, 63 (1948).
126. Paul, A. E., and E. H. Berry, *J. Assoc. Offic. Agr. Chemists*, **5**, 108 (1921).
127. Pepkowitz, L. P., and J. W. Shive, *Ind. Eng. Chem., Anal. Ed.*, **14**, 914 (1942).
128. Perrin, C. H., *Anal. Chem.*, **25**, 968 (1953).
129. Phelps, I. K., *J. Assoc. Offic. Agr. Chemists*, **3**, 306 (1920).
130. Phelps, I. K., *J. Assoc. Offic. Agr. Chemists*, **4**, 72 (1920).
131. Pilch, F., *Monatsh.*, **32**, 21 (1911).
132. Pregl, F., in J. Grant, Ed., *Quantitative Organic Microanalysis*, Churchill, London, 1951.
133. Pregl, F., in H. Roth, Ed., *Quantitative Organische Mikroanalyse*, 7th ed., Springer, Wien, 1958.
134. Radmacher, W., and A. Hoverath, Gluckauf, **96**, 1146 (1960); through *Anal. Abstr.*, **8**, 4258 (1961).
135. Reitsema, R. H., and N. L. Allphin, *Anal. Chem.*, **33**, 355 (1961).
136. Riley, J. P., *Anal. Chim. Acta*, **9**, 575 (1953).
137. Robinson, J. B. D., *Analyst*, **81**, 316 (1956).
138. Rosenthaler, L., *Mitt. Lebensm. Hyg.*, **37**, 215 (1946).
139. Rozental, L., *Roczniki Panstwowego Zakladu Hig.*, **9**, 183 (1958).
140. Sandstedt, R. M., *Cereal Chem.*, **9**, 156 (1932).
141. Scandrett, F. J., *Analyst*, **78**, 734 (1953).
142. Scales, F. M., and A. P. Harrison, *Ind. Eng. Chem.*, **12**, 350 (1920).
143. Schaffer, F. L., and J. C. Sprecher, *Anal. Chem.*, **29**, 437 (1957).
144. Schluter, E. C., Jr., *Anal. Chem.*, **31**, 1576 (1959).
145. Schöniger, W., "Determination of Nitrogen. 1. Determination of Dry Combustion" in C. N. Reilley, Ed., *Advances in Analytical Chemistry and Instrumentation*, Vol. 1, Interscience, New York, 1960, pp. 217–220.
146. Schöniger, W., *Farmaco (Pavia) Ed. Sci.*, **16**, 107 (1961).

147. Schöniger, W., and A. Haack, *Mikrochim. Acta*, **1956**, 1369.
148. Schwab, G. M., and E. Schwab-Agallidis, *J. Am. Chem. Soc.*, **73**, 803 (1951).
149. Scott, W. W., *Standard Methods of Chemical Analysis*, 6th ed., N. Howell Furman, Ed., Vol. I, Van Nostrand, Princeton, New Jersey, 1962.
150. Shedd, O. M., *J. Assoc. Offic. Agr. Chemists*, **10**, 507 (1927).
151. Shelberg, E. F., *Anal. Chem.*, **23**, 1492 (1951).
152. Sher, I. H., *Anal. Chem.*, **27**, 831 (1955).
153. Shirley, R. L., and W. W. Becker, *Ind. Eng. Chem., Anal. Ed.*, **17**, 437 (1945).
154. Simon, W., P. F. Sommer, and G. H. Lyssy, *Microchem. J.*, **6**, 239 (1962).
155. Sobel, A. E., H. Yuska, and J. Cohen, *J. Biol. Chem.*, **118**, 443 (1937).
156. Spears, H. D., *J. Assoc. Offic. Agr. Chemists*, **5**, 105 (1921).
157. Sreenivasan, A., *Indian J. Agr. Sci.*, **4**, 546 (1934).
158. Sreenivasan, A., and V. Sadasivan, *Ind. Eng. Chem., Anal. Ed.*, **11**, 314 (1939).
159. Stebbins, J. H., Jr., *J. Am. Chem. Soc.*, **7**, 108 (1885).
160. Stehr, E., *Microchem. J.*, **4**, 207 (1960).
161. Sternglanz, P. D., and H. Kollig, *Anal. Chem.*, **34**, 544 (1962).
162. Steyermark, A., *Quantitative Organic Microanalysis*, 2nd ed., Academic Press, New York, 1961, pp. 151–187.
163. Steyermark, A., *Microchem. J.*, **3**, 399 (1959).
164. Steyermark, A., personal communication.
165. Steyermark, A., *Quantitative Organic Microanalysis*, Academic Press, New York, 1961, pp. 188–220.
166. Steyermark, A., H. K. Alber, V. A. Aluise, E. W. D. Huffman, J. A. Kuck, J. J. Moran, and C. O. Willits, *Anal. Chem.*, **23**, 523 (1951).
167. Steyermark, A., and B. E. McGee, *Microchem. J.*, **4**, 353 (1960).
168. Steyermark, A., and B. E. McGee, *Microchem. J.*, **5**, 389 (1961).
169. Steyermark, A., B. E. McGee, E. A. Bass, and R. R. Kaup, *Anal. Chem.*, **30**, 1561 (1958).
170. Stubblefield, F. M., and E. E. DeTurk, *Ind. Eng. Chem., Anal. Ed.*, **12**, 396 (1940).
171. Taylor, W. H., and G. F. Smith, *Ind. Eng. Chem., Anal. Ed.*, **14**, 437 (1942).
172. Ulsch, K., *Z. Anal. Chem.*, **25**, 579 (1886); **27**, 73 (1888).
173. VanEtten, C. H., and M. B. Wiele, *Anal. Chem.*, **23**, 1338 (1951).
174. Van Slyke, D. D., and A. Hiller, *J. Biol. Chem.*, **102**, 499 (1933).
175. Vickery, H. B., *J. Assoc. Offic. Agr. Chemists*, **29**, 358 (1946).
175a. Walisch, W., *Trans. N. Y. Acad. Sci.*, **25**, 693 (1963).
176. White, L. M., and M. C. Long, *Anal. Chem.*, **23**, 363 (1951).
177. Wilfarth, H., *Chem. Zentr.*, **56**, 17, 113 (1885).
178. Willits, C. O., M. R. Coe, and C. L. Ogg, *J. Assoc. Offic. Agr. Chemists*, **32**, 118 (1949).
179. Willits, C. O., H. J. John, and L. R. Ross, *J. Assoc. Offic. Agr. Chemists*, **31**, 432 (1948).
180. Willits, C. O., and C. L. Ogg, *J. Assoc. Offic. Agr. Chemists*, **32**, 561 (1949).
181. Willits, C. O., and C. L. Ogg, *J. Assoc. Offic. Agr. Chemists*, **33**, 179 (1950).
182. Willits, C. O., and C. L. Ogg, *J. Assoc. Offic. Agr. Chemists*, **34**, 607 (1951).
183. Winkler, L. W., *Z. Angew. Chem.*, **26**, 231 (1913).
184. Zimmerman, W., *Mikrochem. ver. Mikrochim. Acta*, **31**, 42 (1943).